THE JOURNAL OF DAIRY RESEARCH

EDITED FOR

The Committee of Management

BY

A. T. R. MATTICK, B.Sc., Ph.D.
The National Institute for Research in Dairying, University of Reading

and

J. A. B. SMITH, Ph.D., D.Sc., F.R.I.C.
The Hannah Dairy Research Institute, Kirkhill, Ayr

ASSISTED IN THE SELECTION OF PAPERS FOR PUBLICATION BY

Dr L. BULL (Australia)
Prof. E. L. CROSSLEY (Reading)
Prof. D. B. CUTHBERTSON (Aberdeen)
Prof. I. de BURGH DALY, F.R.S. (Cambridge)
Dr H. P. DONALD (Edinburgh)
Sir PAUL FILDES, F.R.S. (London)
Prof. P. J. J. FOURIE (South Africa)
Prof. R. C. GARRY, F.R.S.E. (Glasgow)
Dr T. GIBSON (Edinburgh)
Dr J. HAMMOND, F.R.S. (Cambridge)
Dr J. O. IRWIN (London)
Dr A. G. LOCHHEAD, F.R.S. (C.) (Canada)
Prof. F. C. MINETT (Houghton)
Prof. W. RIDDET (New Zealand)
Dr K. C. SEN (India)
Sir JAMES A. SCOTT WATSON (London)

VOLUME 22

CAMBRIDGE
THE UNIVERSITY PRESS
1955

Committee of Management

PROF. H. D. KAY, C.B.E., F.R.S. (*Chairman*)
PROF. R. C. GARRY, F.R.S.E.
MR W. H. SENIOR
DR E. MARSDEN
SIR HERBERT HOWARD
DR A. T. R. MATTICK, DR J. A. B. SMITH } *Editors*
MR H. F. BURGESS, M.B.E. (*Secretary*)

Librarian and Editorial Assistant

MISS D. KNIGHT, B.A., F.L.A. Dip.Lib.

Printed in Great Britain at the University Press, Cambridge
(Brooke Crutchley, University Printer)
and published by the Cambridge University Press
London: Bentley House, N.W.1
American Branch: New York
Agents for Canada, India, and Pakistan: Macmillan

10245

CONTENTS

No. 1 (February 1955)

ORIGINAL ARTICLES PAGE

565. A study of variation in twin cattle. III. Growth. J. W. B. King and H. P. Donald 1

566. Studies of the secretion of milk of low fat content by cows on diets low in hay and high in concentrates. V. The importance of the type of starch in the concentrates. C. C. Balch, D. A. Balch, S. Bartlett, Zena D. Hosking, V. W. Johnson, S. J. Rowland and Jill Turner 10

567. The recorded butterfat content of bulk milk from a herd of White Fulani cattle. N. Tasker 16

568. The rate of secretion of milk and fat. G. L. Bailey, P. A. Clough and F. H. Dodd 22

569. A comparison of the diagnostic value of the total and differential cell counts of bovine milk. P. S. Blackburn, Constance M. Laing and D. F. Malcolm 37

570. A medium for the simultaneous enumeration and preliminary identification of milk microflora. Kathleen O. Donovan and J. M. Vincent 43

571. The effect of menadione (2-methyl-1:4-naphthoquinone) on the keeping quality of milk. Constance Higginbottom . . . 48

572. The heat coagulation of milk. G. T. Pyne and Kathleen A. McHenry 60

573. The temperature coefficient of expansion of raw milk. A. L. Short 69

574. Colour changes in heated and unheated milk. II. The whitening of milk on heating. H. Burton 74

575. Colour changes in heated and unheated milk. III. The effect of variation in milk composition on the whitening and browning of separated milk on heating. H. Burton and S. J. Rowland 82

576. The volatile compounds associated with oxidized flavour in skim milk. D. A. Forss, E. G. Pont and W. Stark 91

577. The chemical composition of the colostrum and milk of the sow. Dawn R. Perrin 103

578. The composition of the milk of the blue whale. Margaret E. Gregory, S. K. Kon, S. J. Rowland and S. Y. Thompson . . 108

Reviews of the Progress of Dairy Science:

Section F. Milk-borne disease. J. Smith 113

No. 2 (June 1955)

Original Articles PAGE

579. A study of variation in twin cattle. IV. Emergence of permanent incisor teeth. G. Wiener and H. P. Donald 127

580. The deterioration of milking rubbers. IV. Fat in milk liners. J. H. Cooper 138

581. The deterioration of milking rubbers. V. The effect of rubber composition. Pamela M. Clarke, N. J. Berridge and E. R. Gardner 144

582. Immersion cleaning of milking equipment. C. C. Thiel, P. A. Clough and L. F. L. Clegg with technical assistance from D. N. Akam, Marie Gruber and Elizabeth Hirons 156

583. The effect of steam, hypochlorite and caustic soda used for treating direct-to-can milking equipment, on the bacteriological quality and flora of milk. D. F. C. Carreira, L. F. L. Clegg, P. A. Clough and C. C. Thiel with technical assistance from D. N. Akam, Marie Gruber and Elizabeth Hirons 166

584. Spore-forming organisms in commercial sterilized milk. Elizabeth Grinsted and L. F. L. Clegg 178

585. A phosphatase test for pasteurization of milk using disodium phenolphthalein phosphate as substrate. W. J. Tulloch 191

586. Colour changes in heated and unheated milk. IV. The theoretical background to the reflectance changes in separated milk. H. Burton 200

587. Fluorescence microscopy of fat in milk and milk powder. N. King . 205

588. The influence of the content and composition of protein in milk on the manufacture of cottage cheese. J. H. Henson and T. B. Miller . 211

589. A chemical investigation of the volatile flavour principle of Cheddar cheese. J. C. Dacre 219

590. Quantitative estimation of the amino-acids in Cheddar cheese and their importance in flavour. L. A. Mabbitt 224

Reviews of the Progress of Dairy Science:

Section A. Physiology of dairy cattle. D. C. Edwards . . . 232

No. 3 (October 1955)

Obituary 251

Original Articles

591. The outdoor rearing of calves on grass with special reference to growth rate and grazing behaviour. J. H. B. Roy, K. W. G. Shillam and June Palmer 252

PAGE

592. Studies of the secretion of milk of low fat content by cows on diets low in hay and high in concentrates. VI. The effect on the physical and biochemical processes of the reticulo-rumen. C. C. Balch, D. A. Balch, S. Bartlett, M. Patricia Bartrum, V. W. Johnson, S. J. Rowland and Jill Turner 270

593. Intramammary pressure change in the lactating cow. I. Changes during the milking process. W. G. Whittlestone 290

594. The effect of heat treatment on the lipolytic flora of cream. G. M. El Sadek and T. Richards 295

595. The steam stripping of taints from liquids. III. Batch stripping with particular reference to equilibrium values from batch data. J. K. Scott 302

596. Steam distillation of taints from cream. I. Theoretical considerations and properties of the reference substances, diacetyl and acetoin. F. H. McDowall 311

597. Microscopic appearance of fat on the milk surface as affected by mechanical disturbance of the surface. N. King 328

598. A comparison of the Gerber and Röse Gottlieb methods for the determination of fat in milk. W. P. Crocker, D. I. Jenkins, A. L. Provan, F. J. Macdonald, S. J. Rowland and J. C. D. White . . . 336

599. The lactose-chloride contribution to the freezing-point depression of milk. E. R. Cole and M. Mead 340

600. Further observations on the volatile compounds associated with oxidized flavour in skim milk. D. A. Forss, E. G. Pont and W. Stark 345

601. The effect of salt and of antioxidants on the keeping quality of butter. A. K. R. McDowell 349

602. Experiments in cheese-making without starter. L. A. Mabbitt, Helen R. Chapman and N. J. Berridge 365

603. Development of serologically identified lactobacilli added to cheese made without starter. M. Elisabeth Sharpe 374

604. The importance of lactobacilli in the production of flavour in cheddar cheese. I. Growth of lactobacilli in cheese serum. L. A. Mabbitt and M. Zielinska 377

605. Preparation of a cheese with crystalline rennin. N. J. Berridge . 384

606. A variability study in firmness in cheese using the ball-compressor test. C. P. Cox and Margaret Baron 386

Reviews of the Progress of Dairy Science:

Section B. Bacteriology and mycology applied to dairying. E. R. Hiscox and C. A. E. Briggs 391

COMMONWEALTH BUREAU OF DAIRY SCIENCE
SHINFIELD · READING · ENGLAND

The Bureau is the world centre for collecting information on research and new developments in all aspects of dairying. Its monthly journal *Dairy Science Abstracts* contains both reviews and abstracts.

DAIRY products are acknowledged by all authorities as nutritionally important especially for the young and infirm. Each month readers of *Dairy Science Abstracts* are kept informed of world developments in dairy science.

SCIENCE and technology are the keywords of this age. In *Dairy Science Abstracts* the latest research in physiology, microbiology, chemistry, etc., is reviewed and abstracted.

ABSTRACTS and reviews are essential to keep the busy man well informed. *Dairy Science Abstracts* brings this information to him each month.

Annual Subscriptions, foreign ... ... 50*s.* 0*d.*
U.K. and Commonwealth ... ... 40*s.* 0*d.*
(*for orders sent direct other than trade*)

COMMONWEALTH AGRICULTURAL BUREAUX · CENTRAL SALES BRANCH
FARNHAM ROYAL · SLOUGH · ENGLAND

The Journal of General Microbiology

Editors: B. C. J. G. KNIGHT and A. F. B. STANDFAST

Vol. 12, No. 1 *Contents* *February 1955*

WAINWRIGHT, S. D. and NEVILL, ANN. Modification of the biological effects of ultraviolet irradiation by post-irradiation treatment with iodoacetate and peptone.
WAINWRIGHT, S. D. and NEVILL, ANN. Induction of provariants as a function of dose of ultraviolet radiation.
SALTON, M. R. J. Isolation of *Streptomyces* spp. capable of decomposing preparations of cell-walls from various micro-organisms and a comparison of their lytic activities with those of certain actinomycetes and myxobacteria.
CONNER, R. L. and WAGTENDONK, W. J. VAN. Steroid requirements of *Paramecium aurelia.*
HENDERSON, MOIRA E. K. and FARMER, V. C. Utilization by soil fungi of *p*-hydroxybenzaldehyde, ferulic acid, syringaldehyde and vanillin.
SALL, T. and MUDD, S. A cytological study of *Caryophanon latum.*
DI MENNA, MARGARET E. A search for pathogenic species of yeasts in New Zealand soils.
PROOM, H. The minimal nutritional requirements of organisms of the genus *Bordetella* López.
SINGH, BALBIR and MITCHISON, D. A. Bactericidal activity of streptomycin and isoniazid in combination with *p*-aminosalicylic acid against *Mycobacterium tuberculosis.*
BEECH, F. W. and CARR, J. G. A survey of inhibitory compounds for the separation of yeasts and bacteria in apple juices and ciders.
KLIENEBERGER-NOBEL, E. and CUCKOW, F. W. A study of organisms of the pleuropneumonia group by electron microscopy.
COOPER, P. D. The site of action of penicillin: some properties of the penicillin-binding component of *Staphylococcus aureus.*
SHARPE, M. ELISABETH. A serological classification of lactobacilli.
WHEATER, DOROTHY M. The characteristics of *Lactobacillus acidophilus* and *L. bulgaricus.*
WHEATER, DOROTHY M. The characteristics of *Lactobacillus plantarum, L. helveticus* and *L. casei.*
MANN, S. O. and OXFORD, A. E. Relationships between viable saccharolytic bacteria in rumen and abomasum of the young calf and kid.
AARONSON, S. The purine requirement of *Staphylococcus flavocyaneus.*

The subscription rate is 90*s. net per volume of* 3 *parts, Single parts* 35*s. each, plus postage. Two volumes will be issued each year. Orders should be sent to*

CAMBRIDGE UNIVERSITY PRESS
BENTLEY HOUSE, 200 EUSTON ROAD, LONDON, N.W. 1

THE JOURNAL OF THE

SOCIETY OF DAIRY TECHNOLOGY

17 DEVONSHIRE STREET, LONDON, W.1. *Tel. Langham* 5059

The Journal is issued in quarterly parts, four parts constituting a volume. The parts are usually issued in January, April, July and October each year.

The Subscription rate to non-members of the Society, payable in advance, is £2 net per volume (post free): single numbers, 12*s*. 6*d*. net. Subscriptions and all business enquiries should be sent to the Secretary, Society of Dairy Technology, 17 Devonshire Street, London, W.1.

Each member of the Society receives one free copy of each number. One further copy of each number may (if available) be bought by members for 7*s*. 6*d*.

CONTENTS OF THE OCTOBER ISSUE

Annual Summer Conference: The Development of the Milk Manufacturing Industry in Northern Ireland, by W. A. MANAHAN.
Dairying in Ireland through the Ages, by E. ESTYN EVANS, M.A., D.Sc., F.S.A., M.R.A.I.
Milk Temperature Control from Cow to Creamery, by J. HUTCHINSON.
The Care and Maintenance of Plant in the Dairy, by A. GRAHAM-ENOCK.
The Visit of H.R.H. The Duke of Edinburgh to the National Institute for Research in Dairying, Shinfield, Reading, by J. A. IRVINE.
Some Problems of Cream Processing, by PROFESSOR E. L. CROSSLEY.
Book Review.
Cost Accountancy in the Milk Industry in Northern Ireland, by A. N. LOVESY, F.C.A.
Air Agitation of Milk Storage Tanks.
Sectional Notes.
Some Recent Developments and Researches in Dairy Practice and Technology, by F. H. MCDOWALL.
Personalia.

The Journal publishes not only papers read at meetings of the Society, but also papers on dairy technology by members and non-members

Advertising Offices: 9 GOUGH SQUARE · LONDON · E.C.4

Indian Journal of Dairy Science

Established in 1948 as a medium for the advancement and dissemination of knowledge in regard to dairy science. Published quarterly in March, June, September and December. Subscription £1 sterling or $4.00 in dollar areas. For further particulars apply to the Hon. Joint Secretaries, Indian Dairy Science Association, Hosur Road, Bangalore 1 (India).

Editor: K. C. Sen, D.Sc., F.N.I.

CONTENTS OF VOL. VII, No. 1, MARCH 1954

(Miss) K. R. Lalitha and Noshir N. Dastur. Keeping Quality of Ghee. II. Effect of Storing Butter and Ghee on Vitamin A Content.

M. T. Ragab, A. A. Asker and M. S. Ghazy. Effect of Season of Calving, Dry Period and Calving Interval on Milk Yield and Lactation Period on Egyptian Buffaloes.

H. S. Lakke Gowda. Studies on Jola or Jowar (Andropogon sorghum) as Cattle Fodder. Part II. Chemical Composition of 'White' Variety of Jola at Different Stages of Growth.

I. Sirry and S. D. Rakshy. The Utilisation of Egyptian Ewes Milk in the Manufacture of Danni Cheese.

A. A. Asker, M. T. Ragab and T. H. Kamal. The Effect of Feeding Thyroprotein on the Composition of Milk of Egyptian Buffaloes.

N. B. Shroff, K. M. Narayanan, C. P. Anantakrishnan and K. C. Sen. Studies on Vitamin A in Milk. Part VII. Effect of Processing on the Stability of Vitamin A in Fortified Milk.

VOLUME 22, NO. 1 FEBRUARY 1955

636.2.082.4

565. A STUDY OF VARIATION IN TWIN CATTLE

III. GROWTH

BY J. W. B. KING AND H. P. DONALD

Agricultural Research Council Animal Breeding Research Organisation, Edinburgh

(With 1 Figure)

The purpose of this paper is to present an analysis of data on growth of one-egg and two-egg twin cattle, and pairs of half-sisters, all subject to the same husbandry regime. As foreseen by Hutt(8), one-egg twins have been shown by Bonnier & Hansson(2) and Hancock(6, 7) to offer opportunities for economizing in experimental material when certain simple designs are appropriate. As a result of these studies on growth in body weight, it has been claimed that one-egg twins would be twenty or more times as efficient statistically as pairs of unrelated animals; but since the basis for calculations of efficiency has been the ratio of variances (or variance components) within and between sets of one-egg twins which changes with sample, environment, and character, it is clear that such estimates of efficiency must not be taken too literally(6). To avoid the objection that between-set variances may be inflated by various unknown non-genetic factors, the variances arising within uniformly treated and contemporary one-egg, two-egg, and half-sib pairs may be compared. Such an experiment has the further advantage that it provides a guide to the value of alternatives to one-egg twins or unrelated pairs as material for nutritional and other studies. The experiment from which the following data were drawn was set up with a view to revealing the reasons for the marked concordance of one-egg twins, but in this paper attention is devoted primarily to the relative experimental efficiency of these twins in respect of growth in live weight and height at withers. The original problem of which this is a particular aspect is, however, also considered. One-egg twins will be referred to as MZ, two-egg twins as DZ, and pairs of half-sisters as HZ. A general description and details of this uniformity trial are given in earlier papers(4, 5).

The animals used, all heifers, were of various dairy breeds and crosses and were purchased as calves about 1–2 weeks old; 14–15 pairs of each kind were available for the study of live weight and 13–15 pairs of each for height at withers. These heifers were all raised on the same farm and under the same system of feeding and management. Because the calves were of necessity collected over a period of about 8 months, different pairs were subjected to the changes from indoor to outdoor feeding at different ages. Such differential treatments applied approximately equally to each group, and in any case should not affect the within-pair differences which are of prime concern.

The measure of variability used throughout has been the mean square (M.S.). Where it applies to differences between pairs, it has been calculated from consecutive pairs arranged in order of birth to reduce any effects of season. This procedure would be fully warranted, if, for example, it were known that seasonal effects could be represented by a slow periodic curve, and calves were equally distributed in time. Although it is unlikely that either of these conditions was exactly fulfilled, the method adopted was the most satisfactory available and the resulting M.S. was smaller than that calculated in the conven-

tional manner. For each of the three groups an estimate of the between-pair variance component was made which was then added to the variance component within pairs in order to give the expected M.S. between unrelated but contemporary animals. In this context 'unrelated' means drawn at random from a large population as heterogeneous in respect of breeding as the one from which the present pairs were taken. The estimates of this M.S. therefore may be presumed to be of a size intermediate between a maximum appropriate to heifers drawn at random from the whole cattle population without restriction as to breed or time of birth, and a minimum appropriate to heifers of one breed born at the same time on a single farm.

Since the heifers were weighed and measured regularly it was possible to escape most of the consequences of depending on single or duplicate observations at specific ages (which are subject to considerable experimental error) and to consider the shape of the growth curves, by fitting polynomials and analysing the resulting coefficients. Aitken's (1) method of repeated summation was used. Fitting was carried on as far as the cubic coefficient, regardless of significance of fit. For growth in weight, monthly observations were limited to the period 1–19 months of age. Pregnancy might have affected subsequent weights since services began at 15 months of age. For height at withers, thirteen measurements taken between approximately 3 and 27 months were used. Although for the purposes of curve fitting these measurements were assumed to be equally spaced, there were in fact some minor inequalities in the intervals. Since, however, the two members of a pair were almost invariably measured on the same day, within-pair differences may be safely compared for the different groups. The between-pair differences will be subject to a slight bias, but as these differences are of only subsidiary interest, the bias has been ignored.

RESULTS

A. *Growth in weight*

Means and standard deviations of the polynomial coefficients for each group are given in Table 1. With the aid of the equation

$$\text{Weight} = a_0 + a_1(2x-18) + a_2\left(\frac{6x(x-1)}{1\times 2} - 51x + 153\right) + a_3\left(\frac{20x(x-1)\,(x-2)}{1\times 2\times 3} - \frac{160x(x-1)}{1\times 2} + 544x - 816\right),$$

where x is the number of the month starting from $x=0$ at 1 month of age, representative curves for growth in live weight can be constructed for the MZ, DZ and HZ groups by using the mean values of a_0, a_1, a_2 and a_3 for each group. For height at withers the number of observations was thirteen instead of nineteen as for weight, so that a different equation with different terms following the a coefficients has to be read off from the appropriate table of terminal values and differences of Tchebychef polynomials.

Only twenty-six of the eighty-six sets of coefficients obtained for live weight showed a quadratic term reducing the sum of squares significantly at the 5% level of probability. Nevertheless, sixty-three of the eighty-six cubic terms gave a significantly better fit at the 5% level. Fig. 1 shows the growth curve of the average MZ heifer. Differences in mean values for a_0 arose partly from the initial advantage of the HZ heifers in birth weight and partly from the slightly lower value of a_1 shown by the MZ animals. DZ twins grew as fast as the initially heavier HZ contemporaries. The small and inconsistent a_2 values, both

within and between groups, show that there was no tendency to follow a common parabolic curve of growth over most of the period studied. In contrast the a_3 coefficients were similar in value and sign and arose mainly as a result of the slowing up of growth at about 15 months of age. In order to show the effect of using the a_2 and a_3 coefficients, Fig. 1 includes the straight line obtained from a_0 and a_1 alone.

Table 1. *Value of polynomial coefficients fitted to growth data on one-egg (MZ) and two-egg (DZ) twins and pairs of half-sibs (HZ)*

		a_0		a_1		a_2		a_3	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Weight (lb.)	MZ	417·4	42·1	19·04	1·48	−0·023	0·119	−0·0214	0·0083
	DZ	433·3	55·7	20·39	1·48	0·041	0·098	−0·0248	0·0106
	HZ	467·2	36·2	20·22	1·43	−0·058	0·110	−0·0252	0·0131
Height at withers (cm.)	MZ	109·4	2·53	1·780	0·166	−0·0949	0·0167	0·00318	0·00281
	DZ	110·6	3·52	1·913	0·169	−0·1049	0·0126	0·00250	0·00263
	HZ	111·6	2·62	1·717	0·205	−0·0983	0·0117	0·00390	0·00205

Analysis of variance of the coefficients within pairs yielded the mean squares from which Table 2 has been compiled. The M.S. for MZ pairs are shown as calculated, but those for DZ and HZ pairs have been divided by the M.S. for MZ pairs to bring out the relative variabilities. Thus the M.S. for the a_1 coefficient in DZ pairs was 6·8 times as large, and in HZ pairs 10 times as large, as in MZ pairs.

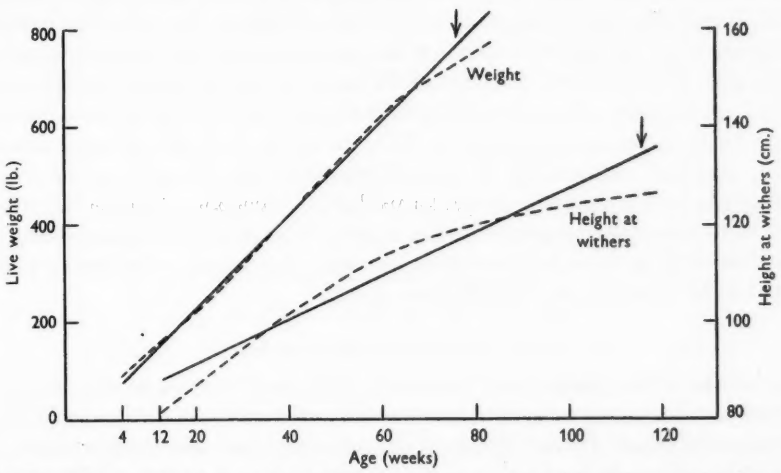

Fig. 1. Curves for growth in live weight and height at withers constructed from the average polynomial coefficients for MZ heifers. —— coefficients a_0 and a_1 used; – – – – coefficients a_0, a_1, a_2 and a_3 used.

For all the coefficients, the DZ pairs were as expected more variable than the MZ pairs, and the HZ pairs more variable than the DZ. In the last column of the table appear the corresponding figures for the between-pair mean squares (calculated as explained earlier from the pooled data for all three groups). These are much larger again. They give an estimate of the error variance *within* pairs that would have been shown had the pairs of calves been made up from single-born contemporary unrelated calves (see above). The conclusion that when rate of growth (a_1) is of interest, one-egg twins would be 21 times as

efficient as unrelated animals probably exaggerates the value of the twins. Since in this material the DZ pairs were 7 times and the HZ pairs 10 times as variable as the MZ pairs, it might be argued from the genetic relationships involved that unrelated contemporary calves within a breed would be about 13 times as variable under comparable conditions of management. As will be seen later this argument is open to objection.

Table 2. *Within-pair variance* (M.S.) *in polynomial coefficients for growth in live weight to* 19 *months of age*

Character	M.S. within MZ pairs lb.^{2} etc.	M.S. relative to M.S. within MZ pairs		
		Within DZ pairs	Within HZ pairs	Between all pairs
a_0	75·1	6·1**	6·9**	23·8†
a_1	0·110	6·8**	10·0**	20·9†
a_2	0·00121	1·6	2·9*	7·5†
a_3	0·00291	1·7	2·3	3·3†
$\log_{10}$ (residual M.S.)	0·0096	0·5	1·6	2·7†

* Ratio significantly greater than one at 5% level.
** Ratio significantly greater than one at 1% level.
† The significance of these ratios has not been calculated.

It is of interest to note that the superior concordance of MZ twins extends, although diminished, to the cubic term—from which it may be inferred that the more precise the problem and the technique, the greater the cumulative merit of one-egg twins for the restricted purposes under discussion. For each column of the table the polynomial coefficients can be placed, with one minor exception, in descending order a_1, a_0, a_2 and a_3, with respect to the relative advantage of MZ pairs. It was suspected that although the similarity of MZ pairs relative to other groups was not very marked for the coefficient a_3, the similarity in the growth curves of MZ twins was not completely accounted for by a cubic equation. Analysis of the residual variation (after transformation by taking logarithms) gave the figures in the last line of Table 2 which show that the MZ twins were not in fact conspicuously superior in this respect. Similarity in behaviour shown by MZ twins does not go as far as daily variations in weight. This was shown by repeated observations at 24 hr. intervals on other MZ twin material.

B. *Growth in height at withers*

The results of this analysis are presented in the same manner as were those of the previous section. All the quadratic polynomial coefficients gave a reduction in the sum of squares significant at the 0·1% level of probability, but only twenty-one out of the eighty-four cubic coefficients gave significant reductions at the 5% level. The means and standard deviations of each coefficient are given in Table 1. The mean heights of the three groups differed in sympathy with the mean body weights, but the coefficients a_1, a_2, a_3 showed values very similar from group to group, so that type of birth had no apparent influence on the course of growth in height up to the age of 27 months. With observations extended to this age, however, the advantage of fitting at least the a_2 coefficients has become very obvious (see Fig. 1).

Table 3 summarizes the results of analysing the variance of the coefficients.

Individual figures differ, but the pattern of ratios shown in the right-hand part of Table 3 conforms with that in Table 2. The two measures of growth used have given similar

results, as would be expected from the high correlation usually found between them in young animals. Scanned vertically in the table the relative variances once more fall (except for DZ pairs) into order of descending magnitude a_1, a_0, a_2 and a_3, although for this character the similarity of MZ pairs relative to the other types of pairs falls off more rapidly. The figures in each row tend to increase from left to right as before, but the ratios tend to be smaller than for live weight.

Table 3. *Within-pair variance in polynomial coefficients for growth in height at withers to 27 months of age*

Character	M.S. within MZ pairs cm.² etc.	M.S. relative to M.S. within MZ pairs: Within DZ pairs	Within HZ pairs	Between all pairs
a_0	0·555	8·0**	3·8*	17·2†
a_1	0·00130	3·6*	12·3**	23·2†
a_2	0·000050	1·1	1·9	2·9†
a_3	0·0000045	1·5	0·9	1·5†

* Ratio significantly greater than one at 5% level.
** Ratio significantly greater than one at 1% level.
† The significance of these ratios has not been calculated.

On the evidence presented here, previous claims for the value of MZ twins in some kinds of experiment involving growth rate can be confirmed. Since the linear component accounts for most of the observed changes in weight up to 18 months of age, the marked advantage of the MZ twins with respect to it suggests that the high heritability found for weight at fixed ages or weight increments over fixed periods applies also to that component. Deviations from a straight line over the period under discussion are probably less heritable and MZ twins less advantageous for their study.

SOURCES OF VARIANCE WITHIN PAIRS

The variation within pairs of the three groups may, as a first approximation (neglecting non-additive effects), be represented by the following simple scheme:

Mean square within MZ pairs: $\sigma^2_{MZ} = e'^2$.

Mean square within DZ pairs: $\sigma^2_{DZ} = e'^2 + \frac{1}{2}g'^2$.

Mean square within HZ pairs: $\sigma^2_{HZ} = e'^2 + \frac{3}{4}g'^2 + m'^2$.

It is assumed here that e'^2, the variance due to environmental effects, is the same within DZ and HZ pairs as within MZ pairs, and that DZ and HZ pairs are drawn from the same population in which the variance due to additive gene effects is g'^2. It is further assumed that HZ pairs, being non-twins, have additional variance, m'^2, not shown by twins. Each HZ pair, having had two dams, may be supposed to be subject to maternal differences of various kinds not arising in twins produced by a single dam, hence the use of m^2 for variance due to maternal effects in an earlier paper (4). It is well to be clear, however, that m'^2, as used here, includes the variance due to any post-natal conditions which are less uniform for non-twins than for twins.

The total variation to be expected between animals which are unrelated is $e'^2 + g'^2 + m'^2$, provided of course they are drawn from a population with an additive genetic variance g'^2, and are born singly within a week of each other. Values for e'^2, g'^2 and m'^2 have been obtained from the three equations given, and each has been expressed as a percentage of

the total variation. Negative values have been regarded as sampling deviations from zero. The results are given in Table 4.

Table 4. *Estimated percentage contribution of* e'^2 *(environment),* g'^2 *(additive genetic effects) and* m'^2 *(maternal effects) to variance* $(e'^2+g'^2+m'^2)$

Character		Percentage of total e'^2	g'^2	m'^2
Growth in weight	a_0	10	90	0
	a_1	8	90	2
	a_2	30	40	30
	a_3	38	51	11
Growth in height at withers	a_0	13	87	0
	a_1	7	39	54
	a_2	50	12	38
	a_3	81	19	0

Heritabilities, as given in the column headed g'^2, tend to be high just as when calculated from the components of variance within and between sets of MZ twins. They may be compared with the heritability values of 86–96% for body weight at 6, 12 and 15 months, and 93–98% for height at withers at the same ages given by Thoele & Hervey (11) and derived only from one-egg pairs. All the figures, however, must be treated with great reserve for the fiducial limits are wide. Errors in estimating e'^2 will directly affect g'^2, and errors in g'^2 will affect m'^2. This is not the only difficulty, for the simple model used does not account for all possible sources of variation. No additional source can be estimated because three estimates have already been obtained from the three equations, but it is possible to decide in which direction these existing estimates will be biased by factors not allowed for.

The variance due to environmental differences (e'^2) may not be constant for the MZ and DZ groups. For instance, the mutual attachment shown by MZ pairs during grazing may result in their having a more uniform environment than DZ pairs. Such an effect would lead to an underestimate of environmental differences between unrelated animals and to an overestimate of genetic differences.

With respect to these genetic differences, it is necessary to consider the effect of neglecting non-additive gene effects in the simple model. If the effects of dominance and epistasis were included, the scheme for the component of the M.S. would be

$$\sigma^2_{MZ}=e^2,$$
$$\sigma^2_{DZ}=e^2+\tfrac{1}{2}g^2+\tfrac{3}{4}d^2+\tfrac{3}{4}i^2,$$
$$\sigma^2_{HZ}=e^2+\tfrac{3}{4}g^2+d^2+\tfrac{15}{16}i^2+m^2,$$

the additional items being

d^2 = variance due to dominance deviations from an additive scheme,
i^2 = variance due to 2-factor epistatic deviations from an additive scheme.

The previous estimate of the environmental component is unchanged. That for the genetic component, however, will now be

$$2(\sigma^2_{DZ}-\sigma^2_{MZ})=g^2+\tfrac{3}{2}d^2+\tfrac{3}{2}i^2,$$

whilst the maternal or non-twin component will be

$$\sigma^2_{HZ}-\sigma^2_{MZ}-\tfrac{3}{2}(\sigma^2_{DZ}-\sigma^2_{MZ})=\sigma^2_{HZ}+\tfrac{1}{2}\sigma^2_{MZ}-\tfrac{3}{2}\sigma^2_{DZ}$$
$$=m^2-\tfrac{1}{8}d^2-\tfrac{3}{16}i^2.$$

Thus the g'^2 as given in Table 4 will overestimate additive genetic variance, and even the total genetic variance ($g^2+d^2+i^2$) if the non-additive portion is at all important. Maternal components (m'^2), on the other hand, will not be seriously affected unless the non-additive portion of the variance is large, when the result will be an underestimate.

There remains one more factor which is possibly more important than all the foregoing. The assumption that all genetic and environmental differences act additively neglects genotype-environment interactions. Should these interactions be important in producing the observed variability between unrelated animals, the use of one-egg twins will overestimate the genetic variance through inclusion of the whole of the variance due to interaction and part of the variance due to the direct effects of environment (10).

These several sources of variation which have been ignored would tend, therefore, to result in overestimates of the additive genetic variation. Whether or not current estimates of g^2, which forms a large percentage of the total variation in twin data, are seriously misleading owing to such omission is at present unknown. The fiducial limits of these estimates are so wide that considerable adjustments could be comfortably accommodated. Taken as a whole, however, the estimates agree reasonably well with estimates of heritability from breeding herds made by other authors for comparable measures of growth. In beef cattle, Knapp & Clark (9) obtained by two methods estimates of 86 and 92% for weight at 15 months of age. Touchberry (12) obtained an estimate of 73% for height at withers in 3-year-old Holstein cattle.

DISCUSSION

It is perhaps well to point out that the so-called 'statistical efficiency' of one-egg twins does not give a comprehensive assessment of their merits for experimentation. The 'statistical efficiency' of groups of animals is given by the ratios of the error variation within each. This is the information supplied by a uniformity trial. Yet in any husbandry experiment using twins and allocating one member of a pair to one treatment and the other member to another treatment, the effect of the treatment is tested for significance against a residual mean square composed not only of a component due to error variation, but in addition a component due to any treatment-set interaction that exists (see also Carter, quoted by Hancock (7)). Although the size of the interaction term is unknown at present, future work may show whether it can be safely ignored.

Provided an estimate of the residual variation for a twin experiment could be given, it would be possible to calculate the number of pairs required to give a certain probability of obtaining a statistically significant result for a given treatment difference. With more than two treatments the experiment has to be confounded in blocks of two. This and any loss of animals complicates the analysis and reduces efficiency (3). Apart from this purely statistical problem, there are other considerations to be borne in mind in assessing the value of twins for experimentation. One is the cost of the twins in relation to the cost of treatment. In general, it seems that the high initial cost of monozygous twins in Britain might be worth while if accommodation were a serious consideration or if the cost of the treatment was high in relation to the normal expense of keeping an experimental cow.

Another consideration is that if genotype-environment interactions occur, generalizations based on a small twin experiment may be ill-advised despite statistical significance of treatment effects. The number of animals used in a study of traits of high heritability could be reduced to the point where sampling inadequacy and restriction of the normal

range of environmental variation could render the interpretation of significant results unusually hazardous.

The preliminary study of resemblance in twins has produced no results incompatible with the assumption that additive genetic effects generate the increasing intra-pair differences that distinguish DZ and HZ pairs from MZ pairs. Within their statistical limitations the estimates of intra-pair variance increase from MZ to DZ to HZ pairs. Nevertheless, the values of m'^2 in Table 4 can be taken as a clear indication that HZ pairs may have a much larger intra-pair variance than would be expected if it were to contain only $e'^2+\frac{3}{4}g'^2$, whilst that of DZ pairs contained $e'^2+\frac{1}{2}g'^2$. The position could well be that e'^2 derived from MZ pairs is not in fact appropriate to HZ pairs but should be increased by an amount which might be equal to m'^2. In this the HZ heifers would resemble ordinary unrelated heifers within a herd more closely than they do twins. The latter may well be a class apart in the sense that when reared together they achieve a uniformity of environment or of response to environment which non-twins under similar conditions do not. If so, heritabilities derived from them are bound to be comparatively high and specific to them. In contemplating this possibility, it is well to bear in mind that if, like e^2_{HZ}, e^2_{DZ} is also greater than e^2_{MZ}, then the estimate of g^2 from MZ and DZ intra-pair variances will be too high and the estimate of m^2 from HZ pairs consequently too low. The genetic mosaicism which follows pre-natal vascular anastomosis, however, may make DZ twins more alike than they would be without this complication and thus tend to cancel the error.

SUMMARY

1. Polynomial coefficients have been fitted to data on growth in live weight to 19 months and on height at withers to 27 months of age shown by one-egg (MZ) and two-egg (DZ) twins and pairs of half-sisters (HZ). The coefficients obtained (a_0, a_1, a_2 and a_3) have been subjected to analysis of variance.

2. For growth in live weight, the ratio of intrapair variances for MZ, DZ and HZ pairs was 1:6·8:10 for a_1, which gives the straight line best fitting the observed curve. Unrelated pairs, it is calculated, would have had an intrapair variance 20·9 times as great as MZ pairs. From the point of view of minimizing the intrapair variance, the advantage of the MZ pairs was usually a little less for a_0, and considerably less for a_2 and a_3.

3. For height at withers, the results were similar to those for weight.

4. The contribution of environmental variance to total intrapair variance increased from a_0 to a_3, while that due to additive genetic effects diminished. Owing to the wide fiducial limits applicable, the results can be accommodated assuming only additive genetic effects in addition to environmental effects as estimated from one-egg twins. The extent to which HZ pairs exceeded the variance expected, however, suggests that this simple assumption may prove inadequate.

The authors are much indebted to Mr D. Anderson who was in charge of the stock, and to Dr St C. S. Taylor who offered constructive criticism of the manuscript.

REFERENCES

(1) AITKEN, A. C. (1933). *Proc. Roy. Soc. Edinb.* **53**, 54.
(2) BONNIER, G. &.HANSSON, A. (1948). *Heredity* **2**, 1.
(3) DICK, I. D. & WHITTLE, P. (1951). *N.Z. J. Sci. Tech.* B, **33**, 145.
(4) DONALD, H. P. (1953). *J. Dairy Res.* **20**, 355.
(5) DONALD, H. P. & ANDERSON, D. (1953). *J. Dairy Res.* **20**, 361.
(6) HANCOCK, J. (1951). *N.Z. J. Sci. Tech.* A, **33** (4), 17.
(7) HANCOCK, J. (1953). *N.Z. J. Sci. Tech.* A, **35**, 189.
(8) HUTT, F. B. (1930). *J. Hered.* **21**, 339.
(9) KNAPP, B. (Jr.) & CLARK, R. T. (1951). *J. Anim. Sci.* **10**, 365.
(10) LUSH, J. L. (1948). *The Genetics of Populations.* Mimeographed, Ames.
(11) THOELE, H. W. & HERVEY, M. C. (1953). *J. Dairy Sci.* **35**, 494 (Abstract).
(12) TOUCHBERRY, R. W. (1951). *J. Dairy Sci.* **34**, 242.

(*MS. received for publication* 6 *June* 1954)

612.664.33:636.2.084

566. STUDIES OF THE SECRETION OF MILK OF LOW FAT CONTENT BY COWS ON DIETS LOW IN HAY AND HIGH IN CONCENTRATES

V. THE IMPORTANCE OF THE TYPE OF STARCH IN THE CONCENTRATES

By C. C. BALCH, D. A. BALCH, S. BARTLETT, ZENA D. HOSKING, V. W. JOHNSON, S. J. ROWLAND and JILL TURNER

National Institute for Research in Dairying, University of Reading

(With 1 Figure)

In the experiment reported in the preceding paper (1) it was shown that falls in milk fat percentage may be expected if cows are given diets which provide a high intake of starch yet have little of the physical property of roughage. Depressions in milk fat percentage occurred when cows were given 4 lb. hay daily and a concentrate mixture containing 50% flaked maize, but not when, with the same intake of hay, the concentrates were given in the form of a dairy cube containing a variety of constituents, but only 5% maize products. Since flaked maize had also formed a high proportion of the concentrate mixtures used in our earlier experiments (2, 3, 4), and as maize starch is known to affect the digestive processes of the reticulo-rumen, it was suggested (1) that the starch of flaked maize, by its effect on digestion, may have played some special role in bringing about the depressions in milk fat content. Accordingly, an experiment has been carried out to determine whether, in diets low in hay, the starch of flaked maize is more effective than the starch of maize meal or of dredge corn (oats and barley) in depressing the milk fat percentage.

METHOD

Fifteen cows from the Institute herd were grouped in blocks of three according to date of calving and the number of previous calvings. Three treatments (A–C) were allocated at random to the cows in each block, giving three comparable treatment groups. The experiment lasted 15 weeks with the following programme of feeding.

Period I (preliminary control), weeks 1–3. All the cows received 16 lb. hay daily, and balanced concentrate mixture *X* (Table 1) at about 4 lb. per 10 lb. milk produced. Additional allowances of concentrate were given to cows early in lactation to ensure that

Table 1. *The concentrate mixtures*

Food	Parts by weight		
	Mixture *X*	Mixture *Y*	Mixture *Z*
Decorticated groundnut cake	3	3	3
Weatings	7	7	7
Flaked maize	10	—	—
Maize meal	—	10	—
Ground dredge corn (oats and barley)	—	—	10

2% of a commercial mineral mixture was added to all concentrates.

the diet was adequate for the standard requirements for protein and energy. Concentrate mixture *X* was used in most other experiments of this series.

Period II (experimental treatment), weeks 4–9. During the first 2 weeks all diets were changed gradually. The hay was reduced to 4 lb. daily and the reduction in nutrients made up by giving 7 lb. extra concentrates. The concentrates given to treatment group A still consisted of mixture *X*, containing 50% flaked maize, but group B received mixture *Y*, containing 50% maize meal, and group C received mixture *Z*, containing 50% dredge corn. Mixture *X* was given at 4 lb., mixture *Y* at 4·2 lb. and mixture *Z* at 4·8 lb. per 10 lb. milk produced, in order to assure an approximately equal intake of starch equivalent.

Period III (final control), weeks 10–13. During the first week the amount of hay was gradually increased to 16 lb. daily and the amount of concentrates reduced, and for groups B and C changed, so that all cows were receiving about 4 lb. mixture *X* per 10 lb. milk produced.

Foods. The hay was of medium quality. The dredge corn was a mixture of oats and barley, in which oats formed 55–60%, and was fed coarsely ground. The foods were sampled on alternate days and bulked for analysis by the standard methods for proximate constituents. Starch was determined by the method of Pucher, Leavenworth & Vickery (5) and cellulose by the method of Norman & Jenkins (6). The constituents of the three concentrate mixtures are given in Table 1 and the composition of the hay and concentrates in Table 2.

Table 2. *Composition of hay and concentrate mixtures* (%)

Food	Dry matter	Crude protein	Ether extract	Nitrogen-free extract	Crude fibre	Ash	Starch	Cellulose
Hay	84·9	6·9	1·2	41·3	29·3	6·2	—	35·3
Concentrate mixture *X*	85·2	16·4	3·1	57·8	4·5	3·4	32·7	7·0
Concentrate mixture *Y*	86·0	16·3	2·9	58·7	4·7	3·4	32·6	9·9
Concentrate mixture *Z*	86·9	15·6	3·0	56·9	7·3	4·1	25·3	12·4

Cows. The thirteen Shorthorn and two Friesian cows had calved 2–12 weeks before the start of the experiment. They were managed in the usual manner in these experiments (4).

Milk. Two 3-day composite samples of milk were taken each week and analysed for fat and solids-not-fat content by standard methods (2).

Statistical analysis. The mean value during the initial control period (weeks 2 and 3) was used as the auxiliary variable in the analysis of covariance for the mean values over weeks 8 and 9.

RESULTS

Milk yield. The mean daily yields of milk from the cows in the three treatment groups are shown in Fig. 1. In the experimental period the yields of the cows fed concentrates containing flaked maize (group A) declined more rapidly than those of cows receiving the other mixtures. When adjustment had been made for initial differences the mean yields in weeks 8 and 9 were 24·5, 28·4 and 30·2 lb. in groups A (flaked maize), B (maize meal) and C (dredge corn) respectively, the differences between the mean values for group A and those for groups B and C were significant (B − A, $P \doteqdot 0{\cdot}05$; C − A, $P \doteqdot 0{\cdot}009$; C − B, $P > 0{\cdot}1$). In the final control period the rate of decline was checked in group A, and by weeks 12 and 13 there were no marked differences between mean values for the three groups.

Fat content of the milk. In Fig. 1 mean values for the milk fat percentage are also given. Comparison of the two control periods shows evidence of the usual decline in early lactation, and, in the experimental period, a pronounced treatment effect for group A and progressively lesser effects for groups B and C are illustrated. The mean observed values for group A were 2·90 in weeks 8 and 9 and 3·57 in weeks 12 and 13, for group B 3·58 and

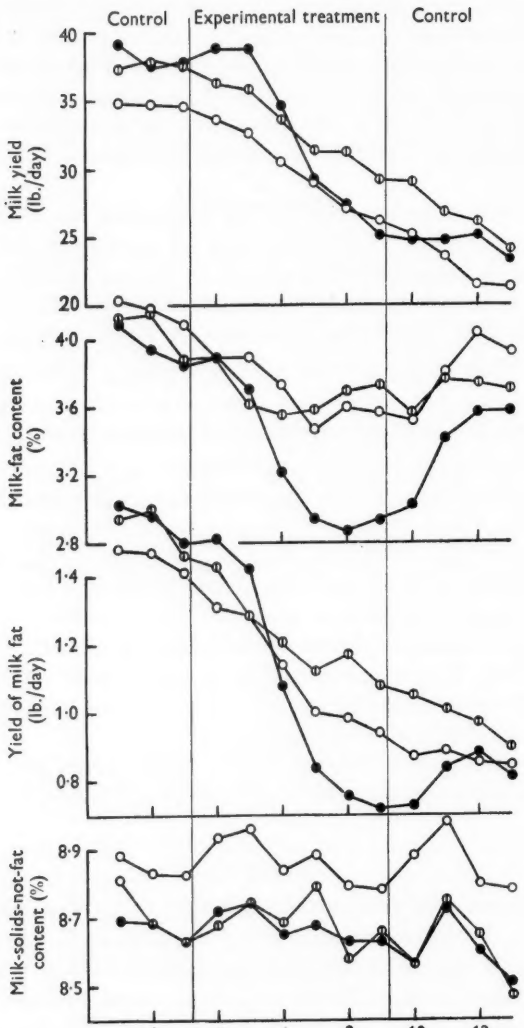

Fig. 1. The mean daily milk yield, fat content, fat yield and solids-not-fat content from groups of five cows receiving daily diet A (4 lb. hay and concentrates containing flaked maize), ●——●; diet B (4 lb. hay and concentrates containing maize meal), ○——○; and diet C (4 lb. hay and concentrates containing dredge corn), ◑——◑, in the period of experimental treatment. During the control periods all groups received daily 16 lb. hay and the concentrate mixture containing flaked maize.

3·94, and for group C 3·71 and 3·73, respectively, giving increases of 0·67, 0·36 and 0·02 for the three groups on returning to the control diet.

The mean milk fat percentages for weeks 8 and 9, adjusted for the initial control period values, were respectively 2·99, 3·50 and 3·70 for treatment groups A, B and C. Thus the depression in milk fat for group A was greater than for groups B and C by 0·51 ($P \doteqdot 0{\cdot}10$) and 0·71 ($P \doteqdot 0{\cdot}03$), and the value for group B was 0·20 below that for group C, which was not, however, significant ($P > 0{\cdot}1$).

Yield of milk fat. The effect of mixture *X* on milk yield and fat percentage was reflected in a marked change in the rate of the decline in fat yield in group A (Fig. 1). In weeks 8 and 9 adjusted values for group A were 0·29 lb. per day less than the values for group B ($P \doteqdot 0{\cdot}02$) and 0·40 lb. per day less than those for group C ($P \doteqdot 0{\cdot}005$). Values for group B were 0·11 lb. per day less than those for group C, but this was not significant ($P > 0{\cdot}1$).

Solids-not-fat content of the milk. In weeks 8 and 9 the mean adjusted solids-not-fat percentages were 8·68, 8·69 and 8·66 in groups A, B and C respectively. Although there were slight fluctuations in weekly values throughout the experiment, these tended to affect each group in the same way (Fig. 1), and clearly in this experiment, unlike the previous experiment (1), the treatments had no effect on the solids-not-fat content of the milk.

Yield of milk solids-not-fat. As a direct result of changes in the rate of decline in milk yield, the adjusted yield of solids-not-fat from cows in group A during weeks 8 and 9 was 0·35 lb. per day below that for group B ($P \doteqdot 0{\cdot}05$) and 0·50 lb. per day below that for group C ($P \doteqdot 0{\cdot}009$). The difference between groups B and C was again not significant ($P > 0{\cdot}1$).

The starch content of the diets. Although the starch content of concentrate mixture *Z* was considerably below that of mixture *X* or *Y* (Table 2), the more liberal rationing of cows in group C ensured that during the experimental period the mean starch intake of group C (5·8 lb per day) was similar to that of groups A (6·0 lb.) and B (6·4 lb.). This is shown in Table 3, together with the mean daily intake of crude fibre.

Table 3. *Mean daily intake of starch and crude fibre in the experimental period*

Treatment group	Main source of starch	Mean daily intake (lb.)	
		Starch	Crude fibre
A	Flaked maize	6·0	2·1
B	Maize meal	6·4	2·2
C	Dredge corn	5·8	2·9

DISCUSSION

The digestibility of starch in concentrate mixture *X* was found previously to be complete (1). It is possible that the digestibility of the starch of mixtures *Y* and *Z* was not quite complete, but because the total intake of starch varied only from 5·8 to 6·4 lb. per cow daily, the amounts of starch digested will have been very similar in each of the three groups. This experiment has, therefore, clearly demonstrated that in diets which contain the same low amount of hay (and hence have similar physical properties) and supply very similar amounts of starch, the nature of the starch determines whether that diet will affect the milk fat percentage. With 4 lb. hay and a concentrate mixture containing 50% flaked maize, the adjusted mean fat percentage was 0·51 below that found with a similar diet

containing maize meal and 0·71 below that found with dredge corn (oats and barley). Comparison of the mean observed values for weeks 8 and 9 with those for weeks 12 and 13 shows that, with these cows, a decline in fat percentage of about 0·4 had occurred during the treatment period on the low hay diet containing maize meal, and a smaller depression on that containing dredge corn. This confirms our earlier suggestion that the special properties of flaked maize were an important factor in the experimental diets with which we have produced depressions in milk fat content. Since a depression occurred with the diet containing maize meal, the possibility is not precluded that in practice depressions of milk fat percentage have been caused by low hay diets having a high content of starch from sources other than flaked maize, especially if excessive amounts of concentrates were given.

The results of our preceding experiment showed that changes in the physical and biochemical processes of the reticulo-rumen occur with diets low in roughage and high in concentrates containing flaked maize (1). It may well be that the special effect of flaked maize, in which the starch granules are ruptured and the starch partly dextrinized by passage between heated rollers, is associated with a changed bacterial metabolism in the reticulo-rumen. The findings of this experiment, and of previous experiments in this series, emphasize the need for more detailed information concerning the effect of diets low in hay and high in concentrates on the types and numbers of rumen bacteria and on their metabolism.

SUMMARY

1. Three comparable groups of cows were used to investigate the effect, on milk composition and yield, of diets consisting of 4 lb. hay daily and concentrates containing 35% weatings, 15% decorticated groundnut cake and 50% of either flaked maize, maize meal or dredge corn (crushed oats and barley) respectively.

2. During initial and final control periods all the cows received 16 lb. hay daily and, per 10 lb. of milk produced, they received about 4 lb. of the concentrate mixture containing flaked maize. During the experimental period of 6 weeks all the cows received 4 lb. hay, concentrates according to milk yield, and an extra 7 lb. of concentrates daily. One group of cows received the concentrate mixture containing flaked maize, a second group the mixture with maize meal and the third group that with dredge corn. The mean daily intakes of starch for the different groups were respectively 6·0, 6·4 and 5·8 lb. per cow.

3. The diet containing flaked maize caused a marked, and that containing maize meal a small fall in the fat content of the milk, but the diet containing dredge corn was without effect. In the last 2 weeks of experimental treatment the adjusted mean fat percentage for the group of cows receiving flaked maize was 0·51 and 0·71 below the percentages for the groups receiving maize meal and dredge corn respectively. The yields of milk and of milk fat were also lower in the group receiving flaked maize than in either of the other groups, but the experimental treatments produced no changes in the milk solids-not-fat percentage.

4. This experiment has shown that when diets low in hay and high in concentrates are given to lactating cows, the type of starch in the concentrates is of great importance in determining the extent of the depression in milk fat percentage. The greater depression with flaked maize than with maize meal or dredge corn is thought to be associated with the effect of the starch on the flora of the reticulo-rumen.

We are again grateful to Mr A. S. Foot and other members of the Dairy Husbandry Department for making the cows and foods available and helping with numerous arrangements for the experiment. We wish also to thank Mr C. P. Cox for guidance on the statistical treatment of the results, Messrs R. Dunster and F. C. Leaver for their care of the animals, Mrs R. Winder and Mr A. W. Wagstaff for the analysis of the milk, and Misses B. K. Allchorne and K. Rhymes for the statistical computations.

REFERENCES

(1) BALCH, C. C., BALCH, D. A., BARTLETT, S., JOHNSON, V. W., ROWLAND, S. J. & TURNER, JILL (1954). *J. Dairy Res.* **21**, 305.

(2) BALCH, C. C., BALCH, D. A., BARTLETT, S., COX, C. P. & ROWLAND, S. J. (1952). *J. Dairy Res.* **19**, 39.

(3) BALCH, C. C., BALCH, D. A., BARTLETT, S., COX, C. P., ROWLAND, S. J. & TURNER, JILL (1954). *J. Dairy Res.* **21**, 165.

(4) BALCH, C. C., BALCH, D. A., BARTLETT, S., HOSKING, Z. D., JOHNSON, V. W., ROWLAND, S. J. & TURNER, JILL (1954). *J. Dairy Res.* **21**, 172.

(5) PUCHER, G. W., LEAVENWORTH, C. S. & VICKERY, H. B. (1948). *Analyt. Chem.* **20**, 850.

(6) NORMAN, A. G. & JENKINS, S. H. (1933). *Biochem. J.* **27**, 818.

(*MS. received for publication* 21 *May* 1954)

637.127.1

567. THE RECORDED BUTTERFAT CONTENT OF BULK MILK FROM A HERD OF WHITE FULANI CATTLE

By N. TASKER

Department of Local Industries, Northern Nigeria

The writer had the opportunity of determining the fat content of morning and evening samples of milk from a herd of White Fulani cattle over a period of 12 months. The notes and tables which follow are the records of this work.

A bulk sample of milk from the herd was tested by the Gerber method for estimation of fat content in milk using the equipment and methods laid down in British Standard Specification No. 696, parts I and II.

The morning sample was tested within 4 hr. of being milked, and the evening sample had been stored overnight at a temperature of approximately 37° F. to prevent souring.

The results of the morning and evening samples have been kept separate for the purposes of tabulation and comparison. A total of 692 samples were tested, of which 343 were morning and 349 were evening milk samples.

During this period January to December 1950, the herd was milked at intervals of 10 hr. (a.m. to p.m.) and 14 hr. (p.m. to a.m.), i.e. at 6.30 a.m. and 4.30 p.m.

The herd is maintained on the Veterinary Department Farm at Vom in the Plateau Province. The elevation above sea-level is 4000–4200 ft.; the rainfall over a period of 13 years averaged 55·46 in. per annum, of which 41·12 in. fell during the months of the year June, July, August and September.

Supplementary feeding of corn, hay and silage is practised from December to April.

HISTORY AND DESCRIPTION OF THE BREED

The White Fulani cow is, by comparison with British dual-purpose cattle, a fairly small beast. It is white haired on a black skin and is black muzzled with dark or black horn tips; the horns curve outward and upward and are very large. The hoof is dark. The Zebu type shows in the hump which, during the rains when there is plenty of grazing, becomes most pronounced. A mature bull weighs about 10½ cwt.; the cows average rather less than 7 cwt., standing about 51 in. high as measured at the shoulder behind the hump. In 1950 the average birth weight of bull calves was 45 lb. and of heifer calves 44 lb.

The White Fulani is found throughout the northern provinces of Nigeria, particularly in the provinces of Kano, Katsina, Bauchi, Adamawa and to a lesser extent in Sokoto, Zaria and Plateau Provinces. These provinces are approximately 198,300 square miles in extent. The very early history of the breed is not really known, because there is no written history of the tribe, and the verbal history which has been passed down from generation to generation speaks only vaguely of the breed travelling along the north coast of Africa and then down into West Africa. According to G. M. Gates (1) the White Fulani is similar to, and possibly a direct descendant of, the Hamitic Longhorn, which is believed to have migrated from Egypt about 2000 B.C.

The breed has produced cattle yielding about 400 gallons of milk in 305 days, but this is outstanding and an average of under 200 is more usual. The yield of the White Fulani cattle maintained by the Fulani cannot be assessed as they do not practise milking the cow as this is understood in European countries, and unless they require the milk for their own household many of the cows only suckle their calf and, of course, there is no record of the yield under nomadic conditions.

In 1950 at the Livestock Improvement Centre at Vom there were ninety-one females in the herd; the average output of milk per cow per annum was 1437 lb. There were sixty-three full-time lactations and the average milk yield in 305 days was 1407 lb.; the average period of lactation in days was, however, only 256.

Much higher yields than this have been recorded from certain cows, and one cow has averaged 382 gallons over five successive lactations; another has given 433 gallons in her second lactation. There are, however, heifers calving down, which go dry after only a few weeks' milking and others which, though continuing in milk for a longer period, give only 70–80 gallons in a lactation period. Selective breeding at the Livestock Improvement Centres is of course increasing the herd average year by year and making available to the Fulani a certain number of bull calves from dams of the higher milking capacities.

RESULTS

The results of the tests carried out are summarized in Tables 1 and 2 below.

Table 1

			a.m. samples			p.m. samples			
	No. of cows in milk	Herd average % fat	Average % fat content	Standard deviation (%)	Coefficient of variation (%)	Average % fat	Standard deviation (%)	Coefficient of variation (%)	Relation of p.m. to a.m. samples (%)
Jan.	48	6·17	5·78	0·386	6·7	6·68	0·218	3·2	+0·9
Feb.	52	6·28	6·14	0·390	6·3	6·67	0·200	3·0	+0·53
Mar.	55	6·03	5·89	0·348	6·0	6·2	0·440	7·1	+0·31
Apr.	58	6·28	6·18	0·575	9·3	6·48	0·500	7·7	+0·3
May	57	6·24	6·07	0·480	8·0	6·48	0·380	5·9	+0·41
June	52	6·50	6·42	0·332	5·2	6·75	0·480	7·1	+0·33
July	54	6·85	6·70	0·316	4·7	6·96	0·35	5·0	+0·26
Aug.	52	6·78	6·80	0·265	3·9	6·77	0·27	4·0	−0·03
Sept.	54	6·85	6·87	0·190	2·8	6·84	0·18	2·6	−0·03
Oct.	51	6·69	6·71	0·425	6·3	6·67	0·435	6·5	−0·04
Nov.	41	6·04	5·99	0·298	5·0	6·12	0·686	11·2	+0·13
Dec.	42	6·08	6·04	0·387	6·4	6·17	0·500	8·1	+0·13
Mean	51	6·39	6·29			6·56			0·27

Table 2 shows the percentage of morning and evening milk samples falling into various fat percentage groups over the year as a whole.

Table 2

	Below 5·5%	5·5% up to and including 5·9%	6% up to and including 6·4%	6·5% up to and including 6·9%	7% up to and including 7·4%	Over 7·4%
a.m. (%)	4·3	20·1	28·8	36·1	10·5	Nil
p.m. (%)	0·57	12·9	20·9	44·4	18·6	2·8

From these tables certain observations can be made. There is a considerable variation in fat content from month to month and from morning to evening samples. There is

a marked change in the relationship of morning and evening samples during the months of August, September and October.

In order to check whether any immediate relationship could be established between fat content and certain climatic conditions, the fat was compared with records of maximum and minimum temperatures and relative humidity. The relation of fat to changes in humidity was checked by comparing fat variations, for periods of eleven days at 2-month intervals, with recorded rainfall and humidity. Tables 3*a–f* show these comparisons. No immediate correlation was found.

Table 3*a*

Date	Relative humidity a.m. (%)	Rainfall (in.)	Fat content a.m.
7. ii. 50	29	Nil	5·3
8. ii. 50	31	Nil	6·7
9. ii. 50	– 16	Nil	6·3
10. ii. 50	– 16	Nil	5·8
11. ii. 50	– 16	Nil	6·2
12. ii. 50	– 16	Nil	6
13. ii. 50	35	Nil	6·3
14. ii. 50	– 16	Nil	6·4
15. ii. 50	– 16	Nil	5·6
16. ii. 50	– 16	Nil	6·3
17. ii. 50	– 16	Nil	6·5

Table 3*b*

Date	Relative humidity a.m. (%)	Rainfall (in.)	Fat content a.m.
7. iv. 50	80	Nil	6
8. iv. 50	62	Nil	6·5
9. iv. 50	61	Nil	6·5
10. iv. 50	57	Nil	5·4
11. iv. 50	84	Nil	5·8
12. iv. 50	– 16	Nil	5·8
13. iv. 50	– 16	Nil	6·4
14. iv. 50	– 16	Nil	6·5
15. iv. 50	83	Nil	5·9
16. iv. 50	57	Nil	7
17. iv. 50	66	Nil	5·9

Table 3*c*

Date	Relative humidity a.m. (%)	Rainfall (in.)	Fat content a.m.
7. vi. 50	90	0·4	6·7
8. vi. 50	79	1·53	6·7
9. vi. 50	75	Nil	6·2
10. vi. 50	85	Nil	6
11. vi. 50	85	0·91	6·5
12. vi. 50	70	Nil	6·5
13. vi. 50	80	0·34	6·5
14. vi. 50	89	0·49	6·3
15. vi. 50	80	0·17	6·7
16. vi. 50	84	Nil	6·2
17. vi. 50	84	0·64	6

During the periods reviewed there are fourteen occasions on which the relative humidity dropped at least 10% in 24 hr. On at least six of these fourteen occasions, the morning fat showed an increase over that of the previous morning; on four occasions the fat showed a decrease in relation to that of the previous occasions and on four further

occasions the fat remained constant. There were ten occasions on which the relative humidity rose 10%. On four of these occasions the fat rose in relation to that of the previous morning, on five occasions the fat decreased in relation to that of the previous morning and on one occasion it remained constant. No immediate relationship was found between changes in humidity and variations in fat content.

Table 3*d*

Date	Relative humidity a.m. (%)	Rainfall (in.)	Fat content a.m.
7. viii. 50	77	Nil	6·7
8. viii. 50	94	Nil	6·9
9. viii. 50	84	0·05	6·7
10. viii. 50	88	Nil	6·9
11. viii. 50	83	0·31	7·3
12. viii. 50	88	Nil	6·6
13. viii. 50	94	0·03	6·7
14. viii. 50	94	0·01	6·8
15. viii. 50	89	Nil	6·7
16. viii. 50	89	0·36	7
17. viii. 50	94	0·15	6·5

Table 3*e*

Date	Relative humidity a.m. (%)	Rainfall (in.)	Fat content a.m.
7. x. 50	79	0·14	7
8. x. 50	78	0·14	5·9
9. x. 50	79	Nil	6
10. x. 50	79	Nil	6·9
11. x. 50	79	Nil	6·8
12. x. 50	79	Nil	6·9
13. x. 50	79	1.00	7
14. x. 50	79	0.01	7·2
15. x. 50	89	Nil	7
16. x. 50	89	0·05	6·7
17. x. 50	79	Nil	6·5

Table 3*f*

Date	Relative humidity a.m. (%)	Rainfall (in.)	Fat content a.m.
7. xii. 50	44	Nil	6·3
8. xii. 50	37	Nil	6·3
9. xii. 50	37	Nil	6
10. xii. 50	44	Nil	7
11. xii. 50	44	Nil	5·8
12. xii. 50	27	Nil	6·2
13. xii. 50	44	Nil	5·8
14. xii. 50	44	Nil	6·5
15. xii. 50	44	Nil	6·5
16. xii. 50	27	Nil	5·5
17. xii. 50	27	Nil	6·8

The fats were then compared with maximum and minimum temperatures prevailing at the time. The period of most stable, and highest, fat content coincides fairly closely with the period when the lowest maximum day temperatures occur and when there is least day/night variation in temperature. It will be noted that on this plateau during the dry season (November to March) very considerable variations between day and night temperatures occur. Table 4 indicates the fat contents and temperatures recorded.

The period July, August, September and October is the period when the maximum amount of grazing is available. During November the grass begins to dry out, and by the end of December and from January onward until mid-April the cattle have frequently to walk long distances grazing as they go. It would appear that the very considerable

Table 4

	a.m. % fat	p.m. % fat	Min. temp.	Max. temp.	Temp. variation max.–min.	Relation of p.m. to a.m. samples
Jan.	5·78	6·68	57·1	81·9	24·8	+0·9
Feb.	6·14	6·67	56·7	82·0	25·3	+0·53
Mar.	5·89	6·20	63·2	86·9	23·7	+0·31
Apr.	6·18	6·48	64·0	86·1	22·1	+0·30
May	6·07	6·48	63·4	83·0	19·6	+0·41
June	6·42	6·75	62·1	80·3	18·2	+0·33
July	6·70	6·96	61·3	73·6	12·3	+0·26
Aug.	6·80	6·77	60·7	72·9	12·2	−0·03
Sept.	6·81	6·86	60·9	76·6	15·1	−0·03
Oct.	6·71	6·67	59·6	81·0	21·4	−0·04
Nov.	5·99	6·12	59·5	81·2	21·7	+0·13
Dec.	6·04	6·17	57·4	81·9	24·5	+0·13

increase in grazing which becomes available during the rains has a marked effect on the cow and leads to improved and more constant fat levels. In the writer's opinion this fact is confirmed because, as can be seen from Table 5, the birth weight of calves follows a similar trend and the maximum birth weight follows the period of maximum available grazing during gestation.

Table 5

Jan.	42·7	Apr.	43·5	July	46·3	Oct.	47·0
Feb.	44·4	May	43·0	Aug.	46·5	Nov.	48·0
Mar.	42·6	June	43·0	Sept.	46·5	Dec.	45·5
	43·2		43·2		46·4		46·8

CONCLUSIONS

From the figures recorded over the year the writer has observed that the fat content of milk from a herd of White Fulani cattle is subject to day-to-day variations in fat content extending over a range of at least 2% and, with individual cows, over a range of 4%.

The variations occurring cannot be directly related to humidity, nor can some of the larger day-to-day variations be ascribed to rainstorms and thunder, because variations are observed during the dry season which are as great as those occurring during the rains.

There is some correlation between temperature and fat content in that the period of the highest fat contents and the least variation between morning and evening corresponds closely to the period of lowest maximum temperatures and least variations between day and night temperatures. This improved stability in the higher fat content coincides with the period of maximum available grazing, and it is this factor which in the opinion of the writer is the most important.

During the dry season what grass there is dries right out; there is not a vestige of green anywhere unless the pasture is artificially irrigated, there is not a cloud in the sky, and the cattle are frequently walked many miles in a day, grazing as they go, and keeping continually on the move to reduce the fly nuisance. During the rains, so vigorous is the growth that grass bursts through everywhere and the cattle just cannot keep it down. In the writer's opinion the evidence afforded by a study of the fat content of the milk of the

cattle and the birth weight of a calf suggests that the nutritional value of the roughage available during the dry season has been over-estimated or that, alternatively, the amount of walking and the interruption to bulk intake caused by walking cattle as they graze has been under-estimated, and that in order to maintain a higher birth live weight and a higher fat content much more extensive supplementary feed must be made available.

It is, however, obvious that the policy of breeding and selection which is being followed at Livestock Improvement Centres will lead to the identification of better milking strains and to improvement in the standard of the herd. It is also obvious that the present practice of the Fulani herdsmen in calving down their cattle in April will lead to the birth weight of their calves being lower than the average for the Government farms.

The foregoing figures and observations did not arise from planned research work; they are the results of tests carried out during the normal working of the Plateau Dairy of which the writer is Manager. Only because of the scarcity or complete lack of any published information on the fat content of this breed in Nigeria are these figures published in the hope that they may be of interest or value to others concerned in milk production or utilization.

I wish to record my thanks to Mr R. S. Marshall, Inspector-General of Animal Health Services, and to Mr J. K. H. Wilde, Assistant Director Laboratory Services, for allowing me to quote from Departmental Records and for permission to publish the figures for fat contents which are the record of tests done on Veterinary Department cattle.

REFERENCE

(1) GATES, G. M. (1952). Breeds of cattle found in Nigeria. *Fm & For.* **11**, 19.

(*MS. received for publication* 21 *April* 1954)

612.664.3:636.2

568. THE RATE OF SECRETION OF MILK AND FAT*

By G. L. BAILEY, P. A. CLOUGH and F. H. DODD

National Institute for Research in Dairying, University of Reading

(With 8 Figures)

The generally accepted theory of the mode of milk secretion during a milking interval (i.e. the interval between consecutive milkings) has been described by Turner (1) in the following way:

'Following the removal of milk at milking time the cycles of milk secretion and discharge from the alveolar cells are rapid, filling the lumina of the alveoli, the ducts and storage space of the duct system and the gland cistern. During this period there is little change in the size of the udder or milk pressure, and the rate of secretion proceeds at a rapid rate. With the continual secretion and discharge of milk there is a gradual rise in udder pressure. The cycles of secretion and discharge slow down.... As secretion continues, milk is discharged which is low in fat.... If the milk is allowed to accumulate in the udder for a long time, the pressure practically inhibits both the secretion and excretion of milk.'

Confirmation of this theory is provided by the indirect evidence of Bartlett (2) and Edwards (3), who compared the ratio of the morning to evening milk yields and showed that the rate of secretion of both milk and fat was less after the long interval, particularly with high-yielding cows or those with small udder capacities. More direct evidence was obtained by the experiments of Ragsdale, Turner & Brody (4) and Johansson (5), who milked cows after intervals of 1–36 hr. These experiments appeared to confirm that the rate of secretion declines with increasing intervals, though the rate of decline is relatively small for the first 12 hr. In addition, the results illustrated the marked drop which occurs in the fat percentage of the milk drawn from the udder as the milking intervals increase. The experiments of Petersen & Rigor (6) also confirmed that increasing the intramammary pressure depresses milk secretion and suggested that secretion ceases at a pressure of about 25 mm. Hg.

Garrison & Turner (7) were the first to throw doubt on the theory that increasing intramammary pressure decreases the fat secretion more than the secretion of water and solids-not-fat. In their experiment both fractions were equally affected by increasing udder pressure; as a result the fat percentages of the milk were similar for all milking intervals. They suggested that the difference between these results and those of Ragsdale *et al.* (4) was due to a different experimental design and that the drop in fat percentage with increasing intervals which was demonstrated by the earlier workers can be explained in the following way: 'The residual milk left in the udder when normal milking is completed is high in fat. This milk is diluted as additional milk is secreted. After short milking intervals the milk obtained would be rich in fat, but as the interval increased, the residual milk would be diluted more and more. This would account for the gradual decline in the fat percentage with increasing interval between milkings....' This supported the hypo-

* The term 'milk' in this paper is used to describe the whole milk as drawn from the cow, while the term 'fat' refers to that fraction of the milk which is measured by the Gerber analysis.

thesis which was first put forward by Bartlett(8) in 1933, that the milk yield at a single milking is distinct from the amount secreted into the udder.

Since that time many data have been collected on the variations in and importance of residual milk; Johansson(9) has reviewed these studies. The importance of residual milk in rate of secretion studies may be summarized as follows. The amount of milk and fat obtained at a milking is equal to the amount secreted in the milking interval plus the residual amount left after the last milking minus the amount left at the current milking. If the amounts of residual milk retained are similar at each milking, then the yield of milk obtained at a milking is equivalent to the amount secreted. But Johansson(9) has shown the marked variations in residual milk which can be expected, and Swanson & Hinton(10), Dodd & Foot(11) and Turner(12) have demonstrated that there is a strong positive relationship between the milk yield and the yield of residual milk. The amount of residual milk for individual cows may be up to 20% of the milk and 50% of the fat present in the gland at the start of milking. Turner(12) has emphasized these considerations and pointed to the bias which must have occurred in the interpretation of the experiments of Ragsdale *et al.*(4) and Johansson(5), who ignored residual milk considerations. In these experiments the cows were milked after milking intervals of various lengths, the order of the intervals being at random. The results of experiments of this design will always be biased, the yields after short intervals being spuriously enhanced and those after a long interval depressed, owing to the inevitable tendency for short intervals to follow longer intervals and vice versa. Because of this the net carry-over of residual milk is greater to the short intervals. Although Turner made no mention of fat secretion, it is to be expected that residual fat effects will be more important because of the extremely high fat content of residual milk.

In a recent comprehensive study of intramammary pressure, Korkman(14) has found that, contrary to the results of the early workers, increasing internal udder pressures inhibit the secretion of fat less than the secretion of the non-fatty fraction of the milk.

OBJECT

The objects of the experiments reported in this paper were to obtain direct evidence of the effects of residual milk on the yield and composition of the milk obtained at subsequent milkings, and also to measure the true rate of milk and fat secretion after these residual effects have been eliminated. A preliminary analysis of some of these results has already been published (Bailey, Clough & Dodd(15)).

EXPERIMENTAL PLANS

The effects of residual milk and the rate of secretion were measured at milking intervals of 3, 6, 9, 12, 15 and 18 hr. The five cows used were all in early lactation, and their mean daily milk yields at the start and end of the experiment were 47·3 and 38·1 lb.

The plan of the first experiment, designed mainly to measure residual effects (Part 1), is given in Table 1. Thirty-seven milkings were carried out for each cow, the order of milking intervals being such that at the completion of the experiment each milking interval had followed a similar interval and also each of the other intervals. With the labour and facilities available, it was necessary to have the same order of milking intervals for all five cows.

There are a number of possible methods which can be used to measure the true rate of milk secretion unbiased by any residual milk effects. Turner (12) removed the residual milk left at the end of milking by milking a second time after an injection of pitocin. In this way he found the relationship between the amount of milk obtained by normal milking and

Table 1. *Plan of experiment* (*Part* 1) *designed to find the effect of residual milk on the yield and composition of the milk obtained at the following milking*

Milking no.	Milking interval (hr.)	Milking no.	Milking interval (hr.)	Milking no.	Milking interval (hr.)	Milking no.	Milking interval (hr.)
1	12	10	15	19	6	28	18
2	12	11	12	20	3	29	6
3	9	12	15	21	18	30	18
4	12	13	9	22	18	31	3
5	6	14	15	23	15	32	9
6	12	15	6	24	18	33	9
7	3	16	15	25	12	34	6
8	3	17	3	26	18	35	9
9	15	18	6	27	9	36	3
						37	12

the residual milk. Using this factor he corrected the results of Eisenreich & Mennicke (13) for residual milk effects. Another method would be to carry out a number of milkings after different intervals and at each milking to remove the residual milk after an injection of pitocin. Under these conditions the yield obtained at the normal milking plus the residual milk would equal the amount secreted. The main disadvantage of both these methods is the practical difficulty of removing all the residual milk. An experiment of the second type was, in fact, carried out as a preliminary to the experiments described in this paper, but in spite of the care taken with the injections, the resulting milk yields and yields of residual milk were very variable. In the light of later work it would appear that all the residual milk was not removed after each milking, although the yields of this residual milk showed the expected relationship with normal milk yield. Because of these difficulties a plan was drawn up which did not involve the direct estimation of residual milk.

The experimental plan (Part 2), which is given in Table 2, was carried out with the same five cows under similar milking conditions as in the first experiment. It is based on the assumption that provided the amounts of residual milk can be kept constant the yield of

Table 2. *Plan of rate of secretion experiment* (*Part* 2) *showing the length of the milking interval at each series of milkings and the number of separate milkings in each series*

Milking series no. ...	1	2	3	4	5	6	7	8	9	10	11	12
Milking interval (hr.)	12	9	15	6	18	3	3	18	6	15	9	12
Nos. of milkings in the series	6	5	6	8	6	12	12	6	10	8	7	6
Nos. of milkings used in analysis	4	3	3	5	3	18		3	5	3	3	4

milk and fat obtained at a milking is *equivalent to* that secreted in the previous milking interval. The same six intervals were adopted, 3, 6, 9, 12, 15 and 18 hr., but in this experiment a series of consecutive milkings was carried out for each interval. The individual milk yields obtained at each milking in a series should be similar because the intervals are equal; therefore it can be assumed that the residual milk left after each milking in a series would also be similar. Thus the milk obtained at each of these milkings can be regarded as the quantity secreted in the previous interval, provided that the initial milkings after

each change-over from one interval to another are not considered. From the plan it will be seen that the number of milkings in a series was greater for the short milking intervals. The reasons for this were that after a change-over residual fat effects persist for a greater number of milkings when the change is made to a short interval. In addition, the milk yields tend to be more variable after short intervals because of the difficulties in stimulating milk ejection. The experiment was planned so that each series was made twice, the order of the series was reversed when each milking interval had been serialized once. This was done in order to reduce the effect of decline in lactation.

Management of the cows while on experiment

The five cows were housed in separate boxes throughout the experiment. In order that milk yields should not be limited by plane of nutrition the rationing of the cows was on a liberal scale. The daily feeding of each cow was 8 lb. hay, 35 lb. kale and 4 lb. of balanced dairy cube mixture per 10 lb. of milk produced. The quantities fed were accurately weighed, the amount being proportional to the length of the following milking interval. With the exception of the 15 and 18 hr. intervals all the food was given at milking time, the concentrated foods just before milking and the kale and hay immediately afterwards. With the two longest intervals all the concentrates were given at milking, but the hay and kale were divided into two parts and the second part fed midway through the period.

RESULTS

The accurate measurement of the rate of secretion of milk in these experiments depended on the extent to which the quantity of milk obtained at a particular milking was equivalent to the quantity of milk secreted into the udder in the previous milking interval. This was ensured by the design of Part 2, but was not exact in Part 1. In the latter the mean effects of the previous milking interval were equalized for each milking interval examined, but the effect was greatest for very short intervals following very long ones and vice versa, but of opposite sign in the two cases. The value of Part 1 lies mainly in the measurements of the effects of variations in the length of the preceding milking interval rather than in the measurements of the rates of secretion.

The data obtained from each cow were analysed separately. This was done because at an early stage in the examination of the results it became apparent that the cows varied in their reaction to the experimental regime. A study of their individual behaviours was therefore more rewarding than a simple estimation of the mean effects.

The statistical treatment of the data of both parts was made as uniform as possible in order to facilitate comparison of the results. Even if a particular regression did not depart significantly from linearity, the non-linear term or terms were included when examination of the other comparable regressions showed these terms to be significant.

The analyses of Part 1 were made on the data from thirty-six of the thirty-seven milking occasions of the trial; the first milking was not included as it was made simply as a control for the second milking. The analyses of Part 2 were made on data from fifty-four of the ninety-two separate milking occasions. It was decided to discard the remaining data, as these were from milkings that occurred at the beginning of each series made at a constant interval. In consequence they were influenced by residual milk effects from the previous series.

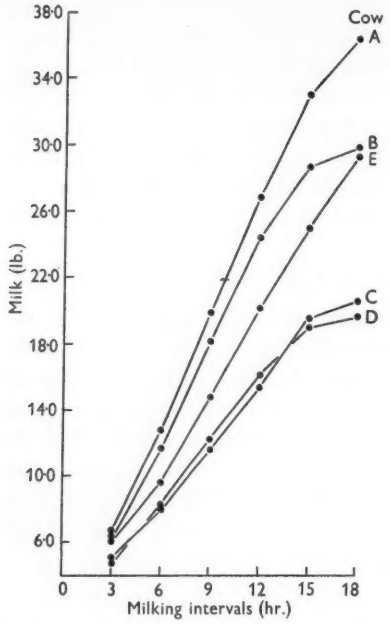

Fig. 1. The milk yields obtained after milking intervals of 3, 6, 9, 12, 15 and 18 hr. (Part 1).

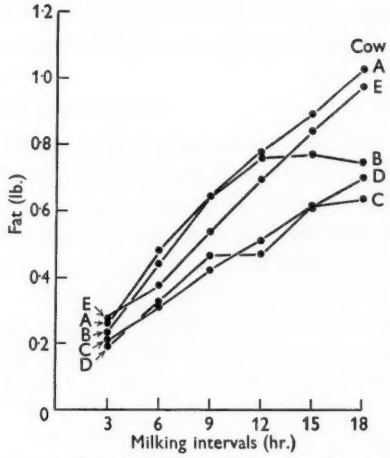

Fig. 2. The fat yields obtained after milking intervals of 3, 6, 9, 12, 15 and 18 hr. (Part 1).

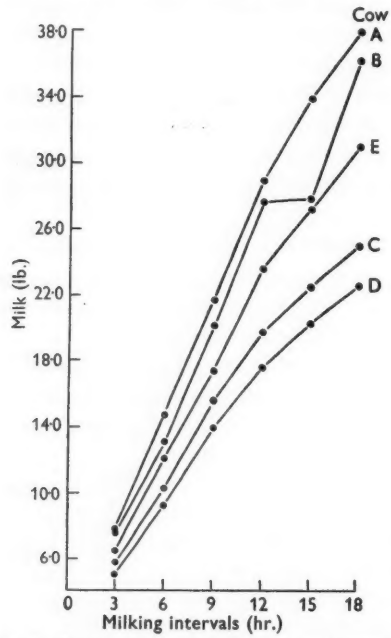

Fig. 3. The mean secretion of milk after intervals of 3, 6, 9, 12, 15 and 18 hr. (Part 2).

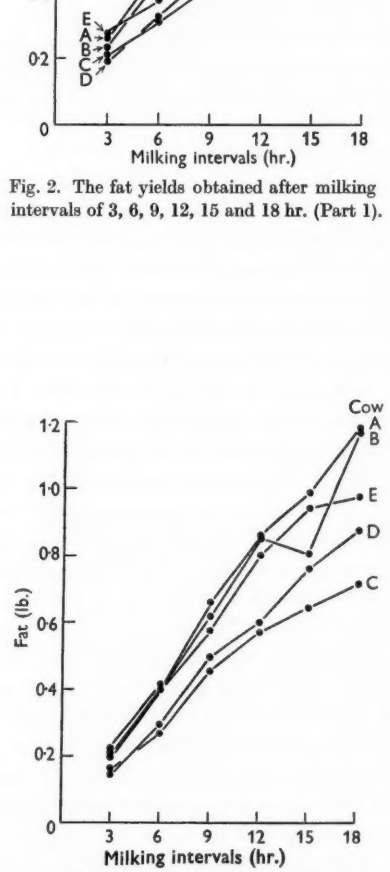

Fig. 4. The mean secretion of fat after intervals of 3, 6, 9, 12, 15 and 18 hr. (Part 2).

The data obtained in both parts of this trial were plotted on squared paper in order to determine the form of the regressions to be examined. In Part 1 the mean yields of milk (Fig. 1) and fat (Fig. 2) were plotted against the milking interval at which they were obtained. In Part 2 (Figs. 3 and 4) the ordinates also show yield of milk and fat; for the reasons already discussed these are equivalent to the mean secretion. Inspection of the four regressions suggested that they were curvilinear, so it was decided to fit them to a quadratic function of the form

$$Y=\bar{y}+B(x-\bar{x})+a\left[(x-\bar{x})^2-\frac{U_3(x-\bar{x})}{\sigma^2}-\sigma^2\right], \tag{1}$$

where Y = the yield of milk fat (lb.), x = milking interval (hours), B and a are unknown coefficients, U_3 is the third moment about the mean and σ^2 is the variance of the x's. In this form the function is orthogonal and permits a distinct separation of B and a. B is the average rate of secretion over the whole period and statistically is an ordinary (total) linear regression coefficient, while the symbol a represents the factors which increase or decrease the secretion rate above or below the average; statistically it is a partial regression coefficient. For convenience the function may be written

$$Y=\bar{y}+B\xi_1+a\xi_2. \tag{2}$$

The rate of secretion can be calculated from the formula by differentiation of y with respect to $(x-\bar{x})$. In Part 1 the rate at any time $=B+2a(x-\bar{x})$. In Part 2 the rate at any time $=B+2a(x-\bar{x})+a34{\cdot}3481$. The coefficient '$a$' is negative, so the rate will be greatest when $x=0$ and will decline as x increases.

A biological background can be attached to the terms of the function. The quadratic term involving 'a' may be regarded as representing the factors which oppose the secretion of milk. There may be several of these, but it is probable that the most important would be the capacity of the udder. This would provide a limit to the quantity of milk that could be obtained at any milking, and as the udder is somewhat elastic the effect of filling the udder will be to build up a resistance to further filling at an increasing rate. This resistance is derived from stretching the walls of the milk cistern and alveoli in two directions, a square measure. This suggests that the use of x^2 in equation (1) is very suitable. The linear term in equation (1) involves B. It may be thought of as representing those factors which promote the secretion of milk, and may be expected to act at a constant rate at least over the short period of this trial; hence a linear term in the equation is mathematically satisfactory.

The numerical solutions of equation (1) were calculated and the results are given for Part 1 milk yield in Table 3, Part 1 fat yield in Table 4, Part 2 milk yield in Table 5, and Part 2 fat yield in Table 6. Because of the design of Part 2 the individual milk and fat yields were equivalent to the amount of milk and fat secreted. It can be seen that although the same milking intervals were used in both parts, the mean values of x and x^2 are different. This was due to the use of unequal numbers of experimental milkings at the different milking intervals in Part 2. The difference in these mean values must be borne in mind when the mean milk and fat yields of the two parts are considered.

The milk data of Tables 3 and 5 show a substantial degree of uniformity. The variation in the mean values of the milk yields simply reflects the differences in the milking capacities of the cows; these differences are reflected in the magnitude of the 'B' coefficients which measure the average rate of milk secretion. The 'a' coefficients show rather more variation,

Table 3. *Milk-yield data and the influence of the length of the previous milking interval. Part* 1

Cow	Mean milk yield $\bar{y}$ (lb.)	Mean milking interval $\bar{x}$ (hr.)	$(\bar{x}^2)$	Coefficient of $\xi_2 x$ a	Coefficient of $\xi_1 x$ B	Mean interval of previous milking $\bar{w}$ (hr.)	$(\bar{w}^2)$	Coefficient of $\xi_2 w$ d	Coefficient of $\xi_1 w$ E
D	13·3	10·5	136·5	−0·0372** ±0·0096	1·0571** ±0·0497	10·5	136·5	−0·0349** ±0·0096	−0·0121 ±0·0497
E	17·4	10·5	136·5	+0·0061 ±0·0100	1·5934** ±0·0437	10·5	136·5	−0·0248** ±0·0100	0·0957* ±0·0437
A	22·5	10·5	136·5	−0·0334** ±0·0107	2·0470** ±0·0134	10·5	136·5	−0·0269** ±0·0107	−0·0463** ±0·0134
B	19·7	10·5	136·5	−0·0583** ±0·0144	1·6575** ±0·0751	10·5	136·5	−0·0193 ±0·0144	0·0225 ±0·0751
C	13·4	10·5	136·5	−0·0151 ±0·0089	1·1003** ±0·0395	10·5	136·5	−0·0151 ±0·0088	−0·0143 ±0·0395

* Significant when P is 0·05. ** Significant when P is 0·01.

Table 4. *Fat-yield data and the influence of the length of the previous milking interval. Part* 1

Cow	Mean fat yield $\bar{y}$ (lb.)	Mean milking interval $\bar{x}$ (hr.)	$(\bar{x}^2)$	Coefficient of $\xi_2 x$ a	Coefficient of $\xi_1 x$ B	Mean interval of previous milking $\bar{w}$ (hr.)	$(\bar{w}^2)$	Coefficient of $\xi_2 w$ d	Coefficient of $\xi_1 w$ E
D	0·460	10·5	136·5	−0·00033 ±0·00028	0·03232** ±0·00277	10·5	136·5	−0·00021 ±0·00028	0·00979** ±0·00277
E	0·615	10·5	136·5	+0·00014 ±0·00044	0·04784** ±0·00204	10·5	136·5	−0·00054 ±0·00044	0·00839** ±0·00204
A	0·678	10·5	136·5	−0·00120** ±0·00036	0·04908** ±0·00173	10·5	136·5	0·00028 ±0·00036	0·01543** ±0·00173
B	0·596	10·5	136·5	−0·00382** ±0·00052	0·03466** ±0·00372	10·5	136·5	0·00051 ±0·00053	0·01465** ±0·00372
C	0·446	10·5	136·5	−0·00067 ±0·00044	0·02949** ±0·00192	10·5	136·5	−0·00005 ±0·00044	0·00953** ±0·00192

* Significant when P is 0·05. ** Significant when P is 0·01.

probably because they were relatively less accurately determined as the curvature of this regression was slight. The 'B' coefficients of Table 3 are smaller than those of Table 5. The reason for this lies in the experimentally induced bias of Part 1, whose operation has been discussed already in the first paragraph of the results. The statistical significance of all but two of the 'a' coefficients of Table 3 and all of Table 5 provides very good evidence of the curvilinearity of the general regression of milk yield on milking interval.

Table 5. *Milk-yield data and the rates of secretion of milk. Part 2*

Cow	Mean milk yield $\bar{y}$ (lb.)	Mean milking interval $\bar{x}$ (hr.)	$(\bar{w}^2)$	Coefficient of $\xi_2 x$ a	Coefficient of $\xi_1 x$ B	Maximum value of y	Value of x when y is maximal
D	13·7	8·7	102	−0·0328** ±0·0159	1·3512** ±0·0490	28·2	29·1
E	16·3	8·7	102	−0·0270** ±0·0100	1·6934** ±0·0483	39·5	53·9
A	20·1	8·7	102	−0·0393** ±0·0041	2·1031** ±0·0388	57·7	64·6
B	18·5	8·7	102	−0·0219** ±0·0100	1·8974** ±0·0480	55·5	72·4
C	12·4	8·7	102	−0·0307** ±0·0064	1·2232** ±0·0330	27·0	29·9

* Significant when P is 0·05. ** Significant when P is 0·01.

Table 6. *Fat-yield data and the rates of secretion of fat. Part 2*

Cow	Mean fat yield $\bar{y}$ (lb.)	Mean milking interval $\bar{x}$ (hr.)	$(\bar{x}^2)$	Coefficient of $\xi_2 x$ a	Coefficient of $\xi_1 x$ B	Maximum value of y	Value of x when y is maximal
D	0·4307	8·7	102	−0·00047 ±0·00037	0·0500** ±0·0017	1·8498	63·4
E	0·5423	8·7	102	−0·00178** ±0·00050	0·0571** ±0·0076	1·1226	26·0
A	0·5945	8·7	102	−0·00081** ±0·00029	0·0662** ±0·0016	2·0100	50·9
B	0·5693	8·7	102	−0·00050 ±0·00071	0·0606** ±0·0031	2·5170	70·6
C	0·3916	8·7	102	−0·00099** ±0·00037	0·0398** ±0·0015	0·8770	30·1

* Significant when P is 0·05. ** Significant when P is 0·01.

The results of Part 2, milk yield, were so consistent that they appeared to be reliable enough to base on them estimates of the capacities of the cow's udders, and of the time interval necessary to fill them completely. This was done by determining the value of x when the rate of milk secretion would become zero ($0 = B + 2a(x - \bar{x}) + a34{\cdot}3481$), and from this value of x determining the concomitant value of y; these x and y values are included in Table 5. The values appear reasonable, but it is important to realize that extrapolation on the scale necessary to obtain these values may involve serious error.

The calculation of the rate of secretion of milk after any milking interval enables further calculations to be made. In many herds the normal interval between evening and morning milking is about 14 hr. With the low-yielding animal, cow C, the rate of milk secretion fell to 0·92 lb./hr. after this interval. This represents a drop of 50% from the maximum rate of 1·84 lb./hr. With a higher yielding cow, cow B, the rate fell from 2·34 to 1·73 lb./hr. in the same time; this is a fall of only 26%. This greater effect with a low-yielding cow is purely fortuitous. Normally, the effect would be greater with high yielders, but in this case the lower yielding animal was a young cow with a small udder which would show intramammary pressure effects more quickly.

The fat data of Tables 4 and 6 are less uniform than the milk data, although again the 'B' coefficients for Part 1 are smaller than those for Part 2; the reason for this is certainly the same as for milk. It is noticeable, however, that the difference between the coefficients is here more marked; this suggests that fat is more influenced by residual milk effects than

is milk. The evidence of general curvilinearity of the fat yield/milking interval regressions is still good, but not so conclusive as the evidence for milk yield. The 'a' and 'B' coefficients can be used in the same way as those for milk yield to calculate the maximum value of y and the corresponding x values; these are given in Table 6. These x values might be thought to correspond with those calculated from the milk data given in Table 5. For cows D and E there is no agreement, but for the other cows the estimates correspond reasonably well. The maximum values of the fat yield may be combined with the maximum values of the milk yield given in Table 5, to estimate the fat percentage at this stage in milk secretion; these estimates are given in Table 7, together with the calculated fat percentage at 8·7 hr. The fat percentage of all except cow E appears to increase with time; this suggests that the rate of secretion of fat falls at a slower rate than the rate of secretion of milk. The discrepant result from cow E is undoubtedly due to errors of the extrapolated values for the time interval before milk and fat would cease to be secreted. Thus the milk would be secreted for 53·9 hr. and the fat for only 26 hr. This large difference accounts for the low value of the calculated fat percentage when milk secretion stopped.

This hypothesis was further tested by calculating the linear regression coefficients of fat percentage on milking interval directly from the experimentally determined data. This could be done only over the range of milking intervals 3 to 18 hr. The results of the calculations are given in Table 7. Only three of the regressions are statistically significant, but they all exhibit a similar trend and afford quite good evidence that fat percentage does, in fact, increase with lengthening milking interval.

Table 7. *The effect on fat percentage of the length of the milking interval*

Cow	Mean fat % at 8·7 hr.	Mean fat % at maximum capacity of the udder	Regression of fat % on milking interval between $x=3$–18 hr.
D	3·14	6·56	0·06902** ±0·01040
E	3·33	2·84	0·01985* ±0·00868
A	2·96	3·48	0·02322** ±0·00506
B	3·08	4·54	0·01548 ±0·00889
C	3·16	3·25	0·01019 ±0·00748

* Significant when P is 0·05. ** Significant when P is 0·01.

The effects of variations in the length of the previous milking interval on the milk and fat yield of the following milking have been studied primarily from the data of Part 1, and the results are given in Figs. 5 and 6. The regressions drawn on the two figures do not exhibit the same form, the regressions of Fig. 5 are decidedly curvilinear, but so far as the regressions of Fig. 6 exhibit any general trend, they appear to be simply linear. In order to preserve the uniformity of the treatment of the data the fat-yield data was treated in the same way as the milk-yield data. This appeared to be best suited by fitting a quadratic function in the form

$$Y=\bar{y}+E(w-\bar{w})+d\left[(w-\bar{w})^2-\frac{U_3(w-\bar{w})}{\sigma^2}-\sigma^2\right], \tag{3}$$

where the symbols have the same meaning as in (1). E and d correspond to B and a and w represents the penultimate milking interval (hr.).

It should be noted that equations (1) and (2) can be combined to form one equation:

$$Y=a\xi_2^x+B\xi_1^x+d\xi_2^w+E\xi_1^w+\bar{y}. \tag{4}$$

As with equation (1) the terms of equation (3) may be regarded as representing biological phenomena. The linear term involving E may be ascribed to the residual milk. It is known that this is proportional to the quantity of milk in the udder before milking, so it must also be proportional to the milking interval. The term involving d is more difficult to attach to any biological factors, because, so far as is known, its existence has not been tested before. It is thought to be concerned with the rate of metabolism of the udder; the evidence for this is discussed later.

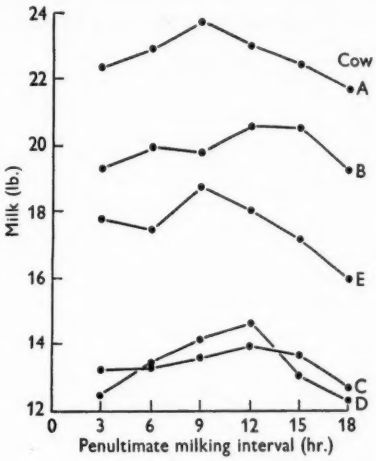

Fig. 5

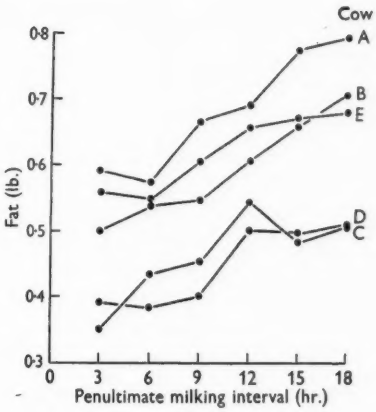

Fig. 6

Figs. 5, 6. The effect on the milk and fat yield obtained at a milking of the length of the penultimate milking interval (Part 1).

The values of the d and E coefficients are given for milk yield in Table 3 and for fat yield in Table 4. The milk-yield data are consistent, and although only three cows gave d coefficients that were statistically significant, the other two cows gave coefficients that revealed similar characteristics. It may be calculated from any of the pairs of coefficients that the maximum benefit to a succeeding milk yield was obtained when the previous milking interval was approximately 10 hr. With cow D the optimum interval was 10·5 hr., and at this interval the gain to the following milk yield was 0·9 lb. A feature of the regressions plotted on Fig. 5 and reflected by the d and E coefficients was that the effect on a following milking of a 3 and an 18 hr. previous milking interval was approximately the same. This may appear to be contrary to the existence of a bias introduced experimentally into the data of Part 1, but it must be realized that the effect of the residual milk exists before the start of a following milking interval, whereas the effect of the rate of metabolism factor does not become operative until the following milking interval is proceeding. Thus the evidence does not, in fact, conflict.

The d coefficients given in Table 4 for fat yield are all without significance, whereas the E coefficients are all significant; by this method of analysis there is no evidence of the real existence of a rate of metabolism factor, but the evidence for the residual milk factor is good. In addition, it is possible to examine the data from Part 1 in a different way. Fig. 7 was prepared showing the variation in the fat percentage when the milking interval

was 3, 6, 9, 12, 15 and 18 hr. and the previous milking interval varied in the same way. This figure shows clearly that the fat percentage increased with the length of the penultimate milking interval, and that the increase was greatest when the penultimate milking interval was 18 hr. and the following milking interval was 3 hr. This affords confirmatory evidence for the existence of the residual milk factor. It has already been pointed out that the relatively large difference in B between coefficients from the fat data of Part 1 and Part 2, compared with the milk data, may be evidence that the effects of the length of the previous milking interval are more for fat than for milk. This was tested by plotting the total milk yield, the total fat yield and the mean fat percentage obtained at each of the

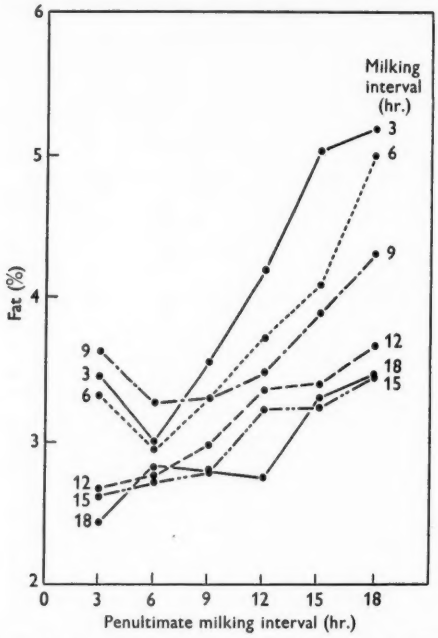

Fig. 7. The effect on the fat percentage of the milk obtained at a milking of the length of the penultimate milking interval (Part 1).

milkings of Part 2 (Fig. 8). From these a study can be made of the relative rate at which the three measurements settle to the mean values at the beginning of each series of milkings at constant intervals. It can be seen that the fat yield usually takes about three milkings to reach a steady value, whereas the milk yield takes only one. This can be seen more strikingly by the reference to the fat percentage. This measure achieves peak values whenever the milking interval is reduced. These high values last for several milkings, confirming that the fat yield is influenced by more of the preceding milking intervals than is the milk yield. It should be noted that when the change in the length of the milking interval is drastic the time taken by the fat to settle to the new mean is longer than when the change in length is only slight.

These findings account for the incomplete success of the results of Part 1 to show how fat yield is influenced by variations in the previous milking interval. The design of the trial involved continuous changes in the milking interval, so that the effect of one interval

became linked with the effect of the two or possibly three preceding intervals. The design was inadequate to permit the separation of these effects, and confused results were obtained.

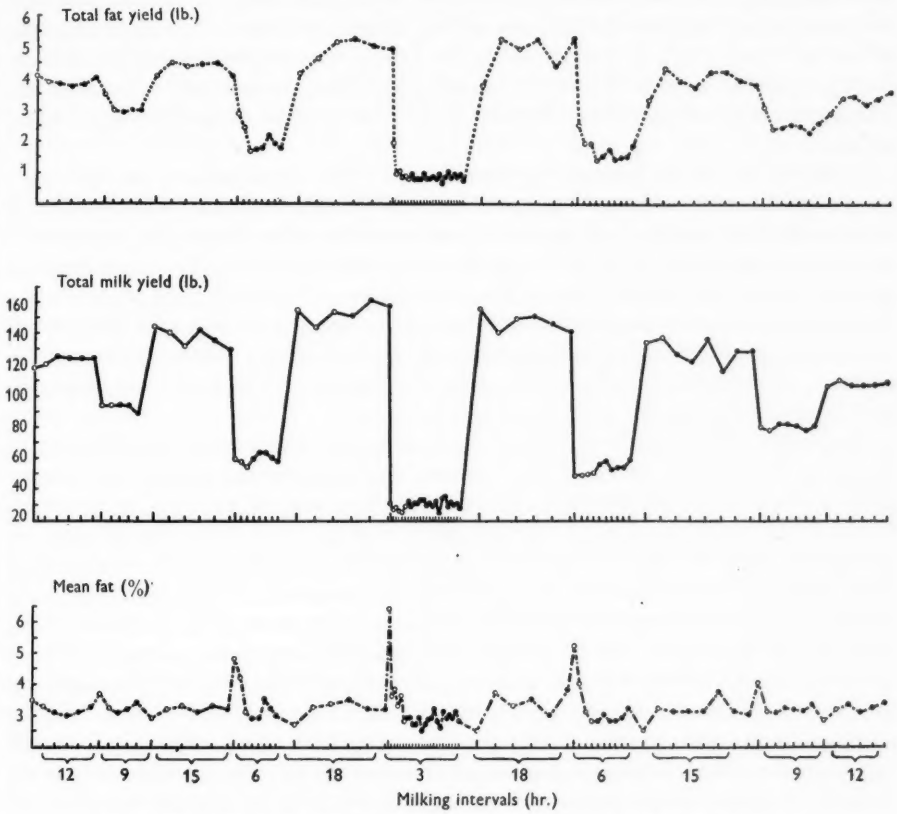

Fig. 8. The yield of fat, yield of milk and mean fat percentage of the milk obtained from the five cows at each milking in Part 2.

DISCUSSION

In common with the results of earlier research the results show that the yield of milk and fat obtained at any one milking depends not only on the length of the interval since the last milking but also on the length of the preceding milking intervals. Further to this, the results here provide detailed measurements of these effects on yields and also show for the first time that two factors are involved; first, the already recognized residual milk factor, and secondly a factor which we have suggested is connected with the metabolic rate of the udder. It has been shown for fat yield that the lengths of more than one of the preceding milking intervals affect the yield at a milking.

The main evidence for a residual milk effect in the results lies in statistical significance of the E coefficients. Other supporting evidence has been given also, and there is no reasonable doubt as to the existence of this residual milk effect in both milk and fat. There

is also little doubt that its effect on yield must be linear within the range of milking intervals used, a finding that is supported by all investigators. If this is accepted then the existence of another factor must be accepted also, for the curvilinearity of the regressions plotted in Fig. 5 is most marked. This second factor apparently becomes increasingly important as the length of the previous milking interval increases. It is known that the rate of secretion which is proportional to the rate of metabolism of the udder declines during a milking interval. If the rate is not, on milking, immediately restored to the maximum but remains depressed for some time, then the effect observed in Fig. 5 would result.

It was not possible to demonstrate the existence of the rate of metabolism factor from the fat-yield results, although this may be due to the relatively much greater effect of residual fat. It is reasonable to assume that up to milking intervals of 18 hr., residual fat is the dominant factor, and by our methods any curvilinearity of the regressions was masked. If we had extended the milking interval up to 36 hr., the rate of metabolism factor might have been demonstrated for fat yield as well as for milk yield. If the rate of metabolism factor does affect milk yields mainly at the beginning of the following milking interval, then the absence of a regular marked decline in yield of a series of milkings at long intervals (see Fig. 8) could be explained.

In the light of these results the difficulties of obtaining a true measure of the rate of milk secretion, uninfluenced by the effect of the length of the preceding milking intervals, are obvious. The method adopted in Part 2 removed the residual milk effect for the reasons discussed in the introduction. However, only part of the rate of metabolism factor was eliminated, and it is difficult to see how this can be done completely. Even when residual milk effects are eliminated there is no absolute single measure of the rate of secretion of a cow at any particular time, reference must always be given to the previous milking interval. In Exp. 2 the rate of secretion during a milking interval was that obtained when the interval followed an equal interval. Thus the curvilinearity of the rate of secretion curves (Fig. 3) was slightly greater than if it had been possible to measure the rate during a single 18 hr. interval uninfluenced by either the residual milk effect or the rate of metabolism effect. However, it is improbable that the bias imparted to results of this type by the metabolism factor is more than trifling with milking intervals of under 18 hr.

The rate of secretion regressions given in Fig. 3 show a slight curvilinearity. That the rate of secretion should decline is reasonable, since the milk is being forced into the ducts and udder sinus against an increasing pressure (Korkman (14)). The relative rates of fat and milk secretion were similar, though the rate of decline in milk secretion was slightly greater than that for fat, and as a result the fat percentages of the milk secreted in 3, 6, 9, 12, 15 and 18 hr. were 2·86, 3·02, 3·19, 3·17, 3·20 and 3·24% respectively. This result is quite different from those of Ragsdale *et al.* (4) and Johansson (5), who made no correction for residual milk, but is in agreement with those of Garrison & Turner (7), whose results were only slightly biased in this way. If, from our results, the fat percentages had been calculated in a similar way to those of Ragsdale *et al.* (4) or Johansson (5) (i.e. from the first milking after each change-over), the mean fat percentage of the six intervals from 3 to 18 hr. would have been 6·34, 4·99, 3·83, 2·81, 2·71 and 2·59% respectively, results which are in line with those of the earlier workers.

The results on fat secretion are in agreement with the recently published results of Korkman (14), and the observed differences in fat percentage between cows milked twice

and thrice daily. Usually no real difference has been measured, but in the experiments of Hansson & Bonnier (16), where small differences could be determined by the use of monozygous twins, there was a slightly lower fat percentage with thrice-daily milking. In addition, Shaw (17) and Smith (18) have demonstrated that if cows are repeatedly milked after short intervals with the aid of oxytocin injections, the yields of milk and fat increase but the fat percentages decrease.

The fact that the results presented in this paper suggest that the milk actually secreted in a long night interval will have a slightly higher fat percentage than the milk secreted in a shorter day interval does not necessarily conflict with the fact that morning milk has a much lower fat percentage than evening milk. The latter difference is probably caused by residual milk effects as suggested by Johansson (19).

A feature of the results of this investigation is the variation in the rate of milk secretion between the individual animals. This variation may arise from two sources, the individuality of the animals and their stage in lactation, and it is profitable to speculate on the implications of each source. It has been observed that the rate of secretion of the different animals varies initially and also in the rate of change. This suggests that managerial conditions which suit one animal might not be best for another. For example, the rate of change for cow C was high, even though her initial rate of secretion was low. Such a cow will evidently give more milk if the period when her rate of secretion is low is kept to a minimum. This can be done by more frequent milking or by equalizing the night- and day-time milking interval, if normally they were unequal. If this were done by milking her last in the evening and first in the morning, it might appear to be a somewhat favoured treatment for a low yielder, yet it might be that relatively she would benefit more from the favour than cows A or B whose rates of decline are so much less.

The variations in the rates of secretion due to stage in lactation were not assessed in this investigation, yet there is no doubt that variations due to this source would be considerable and that individual animals would show different trends depending on the shape of their lactation curve. The general effect of advancing lactation would probably be to reduce the initial rate of milk secretion and to reduce by a relatively greater amount the rate of decline, so that up to a much longer milking interval than was examined here the milk and fat yield/milking interval regressions would appear simply linear.

SUMMARY

1. The effects of variations in the length of the milking interval and of the preceding milking interval were measured for five cows.

2. The yield of milk increased with the lengths and decreased with the square of the lengths of the milking interval and of the previous milking interval.

3. The yield of fat increased with the length of the milking interval and the length of the preceding milking interval and decreased with the square of the length of the milking interval.

4. The biological implications of each of the mathematical terms are discussed.

5. The effect of the preceding interval was through a direct carry-over of residual milk and also by an effect on the metabolic rate of the alveoli throughout the following interval.

6. The rate of secretion of both milk and fat declined with increasing milking interval; there was evidence that the rate of decline for the milk was greater than that for the fat, for the fat percentage of the milk tended to increase.

7. Evidence was presented to show that milk yield in any interval was substantially influenced by only one preceding milking interval, whereas fat yield varied with two or three preceding milking intervals.

We should like to acknowledge the constructive help of Dr J. O. Irwin in interpreting the data and for criticism of the manuscript; in this respect we are also indebted to Dr S. Bartlett, Mr A. S. Foot and Dr S. J. Rowland. Thanks are also due to Dr Rowland for the chemical analysis of the milk, and to Mr C. Machin for preparing the graphs.

REFERENCES

(1) Turner, C. W. (1951). *The Mammary Gland*, p. 236. Columbia, Mo.: Lucas Brothers.
(2) Bartlett, S. B. (1929). *J. agric. Sci.* **29**, 36.
(3) Edwards, J. (1950). *J. agric. Sci.* **40**, 100.
(4) Ragsdale, A. C., Turner, C. W. & Brody, S. (1924). *J. Dairy Sci.* **7**, 249.
(5) Johansson, I. (1949). *Rep. Proc. 12th Int. Dairy Congr. Stockholm*, **1**, 171.
(6) Petersen, W. E. & Rigor, T. V. (1932). *Proc. Soc. exp. Biol., N.Y.*, **30**, 254.
(7) Garrison, E. R. & Turner, C. W. (1936). *Res. Bull. Mo. agric. Exp. Sta.* no. 234.
(8) Bartlett, S. B. (1933). Thesis, University of Reading.
(9) Johansson, I. (1952). *Acta agric. Scand.* **2**, 82.
(10) Swanson, E. W. & Hinton, S. A. (1950). *J. Dairy Sci.* **33**, 383.
(11) Dodd, F. H. & Foot, A. S. (1950). Unpublished data.
(12) Turner, H. G. (1953). *Aust. J. agric. Res.* **4**, 118.
(13) Eisenreich, L. & Mennicke, U. (1950). *Milchwissenschaft*, **5**, 140.
(14) Korkman, N. (1953). *K. Lantbr. Högsk. Ann.* **20**, 303.
(15) Bailey, G. L., Clough, P. A. & Dodd, F. H. (1954). *Nature, Lond.*, **173**, 404.
(16) Hansson, A. & Bonnier, G. (1947). *Acta agric. Suec.* **2**, 311.
(17) Shaw, J. C. (1942). *J. Dairy Sci.* **25**, 1051.
(18) Smith, V. R. (1947). *J. Dairy Sci.* **30**, 703.
(19) Johansson, I. (1940). Reprint of a paper prepared for the 12th World's Dairy Congress, to have been held in Vienna, 1940.

(*MS. received for publication* 30 *April* 1954)

637.128:576.8

569. A COMPARISON OF THE DIAGNOSTIC VALUE OF THE TOTAL AND DIFFERENTIAL CELL COUNTS OF BOVINE MILK

By P. S. BLACKBURN

*Department of Pathology, University and Western Infirmary, Glasgow**

CONSTANCE M. LAING and D. F. MALCOLM

Bacteriology Department, West of Scotland Agricultural College, Glasgow

This work was done to determine whether the differential cell count was superior to the total cell count in the diagnosis of an inflammatory process in the bovine udder.

Early diagnosis of bovine mastitis is essential to enable efficient curative measures to be made before there is permanent damage to the secreting tissue. Formerly, diagnosis was based for the most part on the detection of specific pathogens, the so-called mastitis organisms, in the milk. The total cell content of the milk was not widely used for diagnosing the disease because many workers were of the opinion that in the absence of pathogenic organisms from the milk the presence of leucocytes and other cells in the milk, even in large numbers, was of little or no pathological significance, because such cells were almost always present in milk and their numbers varied according to different factors particularly the stage of lactation. However, Malcolm, King & Campbell (1, 2) claimed that the cell content of mid-lactation milk was a reliable indication of mastitis. They also showed that such milk might contain large numbers of cells even although no mastitis organisms could be demonstrated, a condition referred to as 'non-specific' mastitis. McFarlane, Blackburn, Malcolm & Wilson (3) found that a persistently high cell content in the fore-milk was almost invariably associated with inflammatory changes in the mammary gland as indicated by histological examination of the udder tissue post-mortem. They also demonstrated that the cell content of milk was correlated to a much higher degree with the histological findings post-mortem than the results of the culture tests of the milk. This was due to the fact that in many instances no mastitis organisms were present in the milk, even although its cell content was high and the udder tissues showed pathological changes post-mortem. Further, in many of these cases no pathogens were found in the udder tissues post-mortem. Thus these workers confirmed the earlier findings of Malcolm *et al.* (1, 2) with regard to the occurrence of non-specific forms of mastitis. Chu (4) also came to the conclusion that the high cell count was an indication of mastitis, and McEwan & Cooper (5) employed this test for the diagnosis of mastitis.

In the above investigations attention was focused chiefly on the total numbers of cells present in the milk rather than on the types, but it was thought desirable that information be obtained about the types of cells in the milk and their significance in inflammatory conditions of the udder.

* Now at the Hannah Dairy Research Institute, Kirkhill, Ayr.

MATERIALS AND METHODS

From over 5000 samples of milk which were examined for total cell count and differential cell count and which were studied by culture methods for mastitis organisms 1710 were from cows of which the stage of lactation was known. Of these samples 1271 were from cows in mid-lactation, i.e. cows giving more than 1 gallon of milk daily and not more than 7 months in lactation; 439 were from cows in advanced lactation, i.e. cows giving less than 1 gallon of milk daily and more than 7 months in lactation.

The specimens of milk were examined for total cell content in the Bacteriological Laboratory of the West of Scotland Agricultural College. For this purpose the direct-smear method of Prescott & Breed (6) was used. However, instead of measuring the milk (0·01 ml.) by means of a capillary pipette, a closed platinum loop of standard size was employed. This method of measurement is much more rapid and convenient than the other, and according to the results of an experiment by Malcolm & Smillie (7) there is no significant variation in the count whether the milk is measured by a graduated pipette or a standard size of platinum loop. The smears were stained by Newman's reagent and the cells counted with a microscope giving an oil-immersion field with a radius of 0·1 mm. The area of the field was therefore $\pi \times 0{\cdot}1^2$ sq.mm. As the smear measured 100 sq.mm., there would be $\frac{100}{\pi \times 0{\cdot}1^2}$ fields in the whole smear, i.e. approximately 3000. The amount of milk used in making the smear was 0·01 ml., and therefore 3000 fields would be equivalent to this amount or 300,000 fields to 1 ml. of milk. In estimating the total number of all types of cells present, the cells in thirty fields were counted and the result multiplied by 10,000.

The milk was then examined in the veterinary laboratory, where the samples were treated first with formalin and then smears prepared and stained by the Sudan black–Leishman method of Blackburn & McAdam (8) as described in the previous paper. The cell count was made with a microscope giving an oil-immersion field whose radius was 0·09 mm. The area of the field was therefore $\pi \times 0{\cdot}09^2$ sq.mm. and the factor was $\frac{100}{\pi \times 0{\cdot}09^2} \times 100$, i.e. 400,000. Forty fields were counted and the result multiplied by a factor of 10,000 to give the total cell content per ml.

In making the differential cell counts three tally counters were used, the first for granular cells, i.e. polymorphs, the second for lymphocytes and the third for all other cells (mostly epithelial cells). The count was continued until at least 100 cells had been recorded, and the percentage of the three types of cells thus estimated. This part of the work was done in the veterinary laboratory.

To enable a comparison to be made between the total cell counts obtained by the two laboratories, 313 duplicate specimens of milk were tested, one specimen of the pair being examined by the college laboratory and the other by the veterinary laboratory. The results are given in Table 1. It will be seen from the table that the college laboratory found that 176 of the specimens had cell counts of less than 100,000/ml., and that the veterinary laboratory obtained similar results for 165 of the same specimens. The college laboratory also found that fifty-two specimens had counts between 100,000 and 500,000/ml., whereas the veterinary laboratory obtained similar results for thirty-four of the same specimens. Finally, of the eighty-five specimens which were shown by the college to contain more

than 500,000 cells/ml., seventy-four were found by the veterinary laboratory to give similar results. Accordingly, there was close agreement between the results obtained by the two laboratories, the difference being no greater than could be ascribed to random error. This is confirmed by the fact that if the results of the independent tests of 313 samples are shown separately for each laboratory, as in Table 2, there is practically no

Table 1. *Cell counts of* 313 *duplicate specimens of milk, the duplicate specimens being examined in different laboratories*

College laboratory	No. of specimens	Veterinary laboratory No. of specimens		
		Up to 100,000 cells/ml.	100,000–500,000 cells/ml.	More than 500,000 cells/ml.
Up to 100,000 cells/ml.	176	**165**	11	—
100,000–500,000 cells/ml.	53	9	**34**	9
More than 500,000 cells/ml.	85	1	10	**74**

Table 2. *The independent cell-count groupings of* 313 *duplicate specimens of milk, the duplicate specimens being examined in different laboratories*

	No. of specimens		
	Up to 100,000 cells/ml.	100,000–500,000 cells/ml.	More than 500,000 cells/ml.
College laboratory	176	52	85
Veterinary laboratory	175	55	83

difference between the number of specimens falling into various groups. This is in agreement with the results obtained by Malcolm & Smillie (7), who in a similar experiment found there was no significant difference in the counts obtained by their two laboratories.

The culture tests of the milk specimens were made by spreading approximately 0·01 ml. of the milk samples over half the surface of a 4 in. blood-agar plate. The various colonies developing were examined and typed and subcultures were made. Streptococci were classified according to Lancefield and Minett's groupings and were also typed according to heat resistance and action on aesculin, arginine, sodium hippurate, inulin, salicin, mannitol, litmus milk, etc. Those which did not belong to Lancefield's Group B or C, as determined by precipitin tests, or which did not have cultural or biochemical characters of *Streptococcus agalactiae*, *Str. dysgalactiae*, *Str. uberis* or *Str. bovis* were referred to as 'atypical streptococci'. Staphylococci were typed according to production of pigment, coagulase, haemolysis, and in some cases fermentation of mannitol.

In addition to the above procedure where weekly tests of milk from first lactation heifers were being made, plates of Edwards crystal-violet blood-agar medium (Edwards (9)) were poured, using 0·1 ml. of the specimens as inoculum. Streptococcal colonies which developed in this medium were typed in the same way as those on the direct blood plates.

RESULTS

Of the 1271 samples of milk from cows in mid-lactation (see Table 3) 377 contained specific mastitis organisms, 127 having mastitis streptococci and 250 pathogenic staphylococci. The remaining 894 specimens contained no mastitis organisms. They were either sterile or contained only various saprophytic bacteria, e.g. commensal corynebacteria and micrococci from the udder.

Of the 439 samples from cows in advanced lactation (see Table 4) 101 contained mastitis organisms, forty-five having mastitis streptococci and fifty-six pathogenic staphylococci. The remaining 388 specimens were either sterile or contained no mastitis organisms but only commensal corynebacteria and micrococci.

Table 3. *The average proportion of polymorphs in relation to cell count and absence or presence of mastitis organisms in* 1271 *specimens of mid-lactation milk*

	Average proportion of the cells which were polymorphs, expressed as a percentage	
Total cell count	Mastitis organisms absent	Mastitis organisms present
Up to 100,000 cells/ml.	44	44
100,000–500,000 cells/ml.	51	52
More than 500,000 cells/ml.	55	60

Table 4. *The average proportion of polymorphs in relation to cell count and absence or presence of mastitis organisms in* 439 *specimens of advanced-lactation milk*

	Average proportion of the cells which were polymorphs, expressed as a percentage	
Total cell count	Mastitis organisms absent	Mastitis organisms present
Up to 100,000 cells/ml.	33	49
100,000–500,000 cells/ml.	40	55
More than 500,000 cells/ml.	45	47

It will be seen from Table 3 that in milk of cows in mid-lactation, with increase in the total cell content there was an increase in the average proportion of the cells which were polymorphs. This applied whether mastitis organisms were present or not. Further, the average proportion of polymorphs was appreciably lower with counts of less than 100,000 cells per ml. than with high cell counts, the average proportion in the former being 44% whether mastitis organisms were present or not; and in the latter 51–60%. This is consistent with findings of Malcolm *et al.* (1, 2) and McFarlane *et al.* (3) that in mid-lactation milk, cell counts of less than 100,000 per ml. indicate that the specimens are probably from mastitis-free cows even although mastitis organisms are present; and that higher cell counts indicate inflammatory changes in the udder. With the latter a rise in the proportion of polymorphs is to be expected. The table also shows that there was on the whole no marked difference in the average proportion of polymorphs in milk of the same total cell content whether mastitis pathogens were present or not. However, in specimens containing more than 500,000 cells/ml., the proportion of polymorphs was rather higher where mastitis organisms were present. This appeared to be due to the fact that a number of these specimens were derived from clinical cases of mastitis, and such specimens have extremely high polymorph proportions.

In samples from cows in advanced lactation (see Table 4) the average proportion of polymorphs in the specimens containing no mastitis organisms increased as the cell count rose, but there was no similar consistent trend where mastitis organisms were present. The average proportion of polymorphs was, however, invariably lower in the

absence of mastitis organisms than in their presence, and this was more marked where the cell count was less than 500,000/ml. In this connexion it must be kept in mind that in advanced lactation, milk even from mastitis-free cows has as a rule a high total cell count (more than 100,000/ml.) owing to the presence of large numbers of epithelial cells, shed from the mammary tissue in the process of involution. Thus such advanced-lactation specimens as showed no mastitis organisms, could be for the most part from mastitis-free cows, particularly where the cell count was not excessively high, i.e. not more than 500,000/ml. One would expect in such mastitis-free specimens that owing to the presence of large numbers of epithelial cells the average proportion of polymorphs would be low. On the other hand, the advanced-lactation specimens which contained mastitis organisms would often be derived from cows suffering from mastitis, and as a result of inflammatory conditions would show much higher polymorph proportions.

As a whole, the specimens from cows in advanced lactation and free from mastitis organisms had appreciably lower polymorph proportions than those of similar specimens from cows in mid-lactation. This, as previously mentioned, would be due to the larger content of epithelial cells in advanced-lactation milk. In milk containing mastitis organisms and presumably in many cases drawn from cows having mastitis, the polymorph proportion of advanced-lactation specimens, where the cell count was more than 500,000/ml., was also appreciably lower than that of mid-lactation specimens. This was no doubt due to the increased numbers of epithelial cells in the advanced-lactation milk. There was, however, no marked difference between the polymorph proportions of advanced-lactation and mid-lactation milk in specimens which contained mastitis organisms and had lower cell counts than 500,000/ml. With such advanced-lactation specimens the fact that the cell count is less than 500,000/ml. shows that involution is not proceeding rapidly and that there is not therefore excessive shedding of epithelial cells. For this reason the effect of mastitis lesions in raising the polymorph proportion of the milk is more evident in these specimens than in those of higher cell count.

CONCLUSIONS

It would appear from the foregoing results that the differential cell count possesses little or no advantage over the total cell count in the diagnosis of mastitis. However, the finding of a relatively low proportion of polymorphs, say less than 45%, in milk of low total cell content (less than 100,000/ml.) provides additional evidence that the milk is from a mastitis-free cow, whatever the stage of lactation. In this case the differential cell count is valuable as a confirmatory test to the total cell count, expecially where doubts arise owing to the presence of mastitis organisms in the milk. In addition, as the proportion of polymorphs tends to be lower in late-lactation milk due to the presence of large numbers of epithelial cells, the differential cell count may be of value as a confirmatory test in milk of high cell content where the stage of lactation is unknown. In such a case a low proportion of polymorphs may indicate freedom from inflammation, especially in the absence of mastitis organisms.

SUMMARY

Total and differential cell counts were obtained for 1710 samples of milk taken from cows in which the stage of lactation was known. A bacteriological examination of the samples was also made. The total and differential cell counts considered together showed no

marked advantage over the total cell count alone in the diagnosis of mastitis, except in milk of late lactation.

This work was aided by a grant from the Agricultural Research Council.

REFERENCES

(1) Malcolm, J. F., King, Catherine, W. & Campbell, Mary M. (1942). *Proc. Soc. agric. Bact.* 30.
(2) Malcolm, J. F., King, Catherine W. & Campbell, Mary M. (1944). *Vet. Rec.* **56**, 227.
(3) McFarlane, D., Blackburn, P. S., Malcolm, J. F. & Wilson, A. L. (1949). *Vet. Rec.* **61**, 807.
(4) Chu, S. J. (1949). *J. comp. Path.* **59**, 81.
(5) McEwan, A. D. & Cooper, Margaret B. (1947). *Vet. Rec.* **59**, 655.
(6) Prescott, S. C. & Breed, R. S. (1910). *J. infect. Dis.* **7**, 632.
(7) Malcolm, J. F. & Smillie, D. M. (1942). Unpublished data.
(8) Blackburn, P. S. & McAdam, I. (1954). *J. Dairy Res.* **21**, 31.
(9) Edwards, S. J. (1933). *J. comp. Path.* **46**, 211.

(*MS. received for publication* 6 *November* 1953)

637.128.576.8

570. A MEDIUM FOR THE SIMULTANEOUS ENUMERATION AND PRELIMINARY IDENTIFICATION OF MILK MICROFLORA

By KATHLEEN O. DONOVAN and J. M. VINCENT

Milk Research Laboratory, School of Agriculture, University of Sydney

(With 1 Figure)

In studying bacteria isolated from milk, it is obviously desirable to obtain information on their growth and the changes they effect in that medium. Sterilized milk containing litmus, or other indicator, is widely used for this purpose, and the results can be put on a quantitative basis by random picking from a counted plate of isolated colonies, each into a separate tube of milk. Considerable time and materials would be saved if the agar medium itself were to reveal changes correlated with those brought about in milk by the same organisms.

In attempting to develop such a medium, it was felt that, as a first requirement, it should give the same estimate of the total viable population as the standard glucose-tryptone skim-milk agar (1); further, that the sugar should be lactose, as in milk, and that indicators should be incorporated to reveal changes on both sides of neutrality. It would also be an advantage if the precipitation and hydrolysis of casein could be demonstrated.

EXPERIMENTAL

Glucose tryptone skim agar was selected as a starting-point because it gives higher total counts (2, 3, 4), supports the growth of a wider range of organisms (3, 5)—including the thermodurics (4, 6)—and gives larger individual colonies (2, 3, 4) than any comparable medium.

Sugar. It was found that lactose could be substituted for glucose without any decline in count.

Indicator. Although litmus has the advantage of a wide range as a pH indicator (pH 5–8), it is difficult to standardize and, for the present purpose, has the disadvantage that it also acts as a redox indicator. A mixed indicator seemed to hold most promise and tests of several combinations led to the selection of brom-cresol purple and phenol red, covering a pH range of 5·2–8·4, through yellow, red to purple.

Casein clot and hydrolysis. 1 ml. sterile skim milk, added to 10 ml. medium, provided the right degree of opacity to detect casein precipitation and digestion, whilst still permitting the easy counting of colonies.

Complete medium (*double indicator agar*, D.I.A.). The complete medium has this composition: agar, 15 g.; bactotryptone, 5 g.; Lab. lemco beef extract, 3 g.; lactose, 1 g.; brom-cresol purple powder, 0·05 g.; phenol red powder, 0·12 g.; distilled water, 1 l.; final pH, 6·8.

The constituents are dissolved by heat, filtered hot through cotton-wool to ensure clarity and tubed in 10 ml. 'deeps'. One ml. of sterile skim milk is incorporated evenly in each plate at the time of pouring.

Tests of method. Total counts on both media were compared for twenty-eight raw and twenty-six laboratory-pasteurized samples after incubation at 30° C. for 3 days.

pH changes, and casein precipitation and hydrolysis observed in the vicinity of individual and randomly selected colonies were compared with the reactions of the same colonies after picking into litmus milks and incubating at 30° C. for 3 days, except where otherwise stated.

RESULTS

Fig. 1 shows the appearance of a representative plate with colonies that cause clotting and proteolysis, together or separately. No indication of the distinct colour change is, however, given.

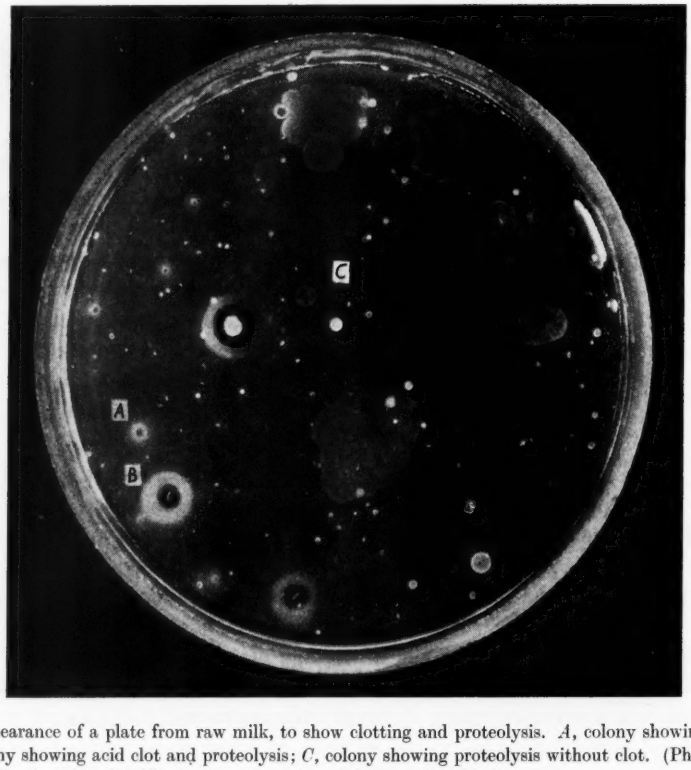

Fig. 1. Appearance of a plate from raw milk, to show clotting and proteolysis. *A*, colony showing acid clot; *B*, colony showing acid clot and proteolysis; *C*, colony showing proteolysis without clot. (Photograph by Mr S. Woodward-Smith, Illustrations Department, University of Sydney.)

Comparison with glucose-tryptone skim-milk agar. A preliminary test with samples of raw and laboratory-pasteurized milk failed to show any significant difference between counts in the two media. A more detailed comparison is shown in Table 1.

There was no significant difference between the media for counts of the thermoduric bacteria of laboratory-pasteurized milk, even when plates up to the maximum limits of Wilson (7) were included. On the other hand, the new medium was more affected by the crowding of organisms from raw milk, so that plates counted to the same limits gave a significantly lower average (about 12%) on D.I.A. This small difference was, however,

virtually removed by restricting the countable plates to those having 30 to 300 colonies, the most suitable range according to Wilson.

Comparison between predicted and actual reactions in litmus milk. Data for raw and laboratory-pasteurized milks are given in Table 2.

Table 1. *Comparison of total viable counts on glucose-tryptone skim-milk agar and double indicator agar*

Type of milk	Maximum colonies on counted plate	Mean colonies* (org./ml.) G.T.S.A.	Mean colonies* (org./ml.) D.I.A.	Statistics† t	Statistics† D.F.	Statistics† P
Laboratory pasteurized	500	109,000	117,000	1·7	25	0·1
Raw	500	264,000	231,000	4·31	27	< 0·01
	300	273,000	257,000	1·65	27	0·1

* Geometric means † Based on log counts.

Table 2. *Predictions of reaction in litmus milk from behaviour of isolated colonies on double indicator agar*

Type of milk	Predictions	Reactions of individual colonies: Acid	Alkali	Proteolysis	Clot	No clot
Laboratory pasteurized	Total number	387	19	8	0	381
	Number correct	356	18	8	0	351
	Percentage correct	92	95	100	—	92
Raw	Total number	356	150	20	87	245
	Number correct	303	64	20	59	176
	Percentage correct	85	43	100	68	72

Results with these laboratory-pasteurized milks were very reliable. However, the estimate of clotting ability was less satisfactory in a further study of 360 colonies. In the latter case, 43% of the pickings selected as clotters failed to clot litmus milk in 3 days, although continuation of the litmus milk test for 14 days reduced the discrepancy to 14%. On the other hand, 'no clot' forecasts were less often in disagreement with the 3-day litmus result (24%) than with the 14-day test (44%). The overall disagreement was much the same with 3- and 14-day litmus milks (33 and 29% respectively).

The reliability of predictions for raw milk decreased in the order: proteolysis (good), acid reaction (fair), clotting power (poor) and alkali reaction (very poor). In 122 cases where the pH change was wrongly predicted, re-streaking from the growth in litmus milk to a fresh plate of D.I.A. resulted in every case in a reaction that agreed with the litmus milk and not with the original prediction. The error in prediction in these cases must be attributed, therefore, not to differences between the agar and milk as media for the organisms' growth, but to interference between colonies on the counted plate. This has been due chiefly to a masking of acid reaction by vigorous growing alkali producers, an effect that shows up particularly in crowded plates (Table 3).

Table 3. *Predictions of reaction in litmus milk from behaviour of eighty isolated colonies from overcrowded plates of double indicator agar (raw milk)*

Predictions	Reactions of individual colonies: Acid	Alkali	Proteolysis
Total number	16	64	7
Number correct	16	0	6
Percentage correct	100	0	86

DISCUSSION

Double indicator agar can profitably replace glucose-tryptone skim-milk agar for plate counts of laboratory-pasteurized and raw milks, provided that for the latter, counts are restricted to plates containing not more than 300 colonies. Whereas the information given by glucose-tryptone skim-milk agar stops short at the total viable count, the new medium provides, with no more trouble, a considerable amount of information about the biochemical properties of the bacterial population and indicates the kind of changes its members are likely to bring about in milk itself. This is particularly true of acid-producing and proteolytic bacteria in both raw and laboratory-pasteurized milk, as well as of alkali producers in the latter.

The estimate with this medium is likely to include a large proportion of colonies wrongly classified as alkali producers in raw milk. This is chiefly due to interference between colonies having different reactions, an effect most likely to be encountered amongst the more diverse and biochemically more active population of a raw milk. The effect will naturally be more serious in crowded plates, and, in fact, a spreading alkali-producing colony can eventually dominate the reaction of the whole plate (as, for example, in Table 3). Interference by acid-producing colonies, which are usually much smaller, is considerably less.

Interference between colonies can be avoided by using plates containing 100 colonies or less for the classification of individual colonies. (The plate in Fig. 1 is at the upper limit for safe classification of individual colonies.) However, plates containing larger numbers of colonies can still give a general impression of the biochemical properties of the dominant bacteria. For example, a clear, pale yellow colour is typical of the weaker acid production of the thermoduric population of pasteurized milk. On the other hand, a bright yellow, cloudy appearance is more typical of raw milks with their vigorous acid-producing population and consequent casein precipitation. The appearance of excessive proteolytic and alkali producers (the last interpreted with some caution) can also provide useful indications of departure from normality.

In conclusion, it can be claimed that the proposed medium has some advantages over glucose-tryptone skim-milk agar even for raw milks, and that it is of particular value in permitting a preliminary classification of thermoduric organisms. The medium is also valuable for the maintenance and study of pure cultures of milk organisms, and for detecting contaminants and biochemical variants.

SUMMARY

A medium has been developed that permits the viable count of milk bacteria to be combined with the determination of biochemical properties likely to be important in milk itself. This has involved the modification of standard glucose-tryptone skim-milk agar by incorporation of two indicators to detect alkali as well as acid production, substitution of lactose for glucose, and increasing the quantity of skim milk for the detection of proteolysis and casein precipitation. The medium has proved particularly valuable in the study of the thermoduric flora of pasteurized milk. The phenomenon of casein precipitation is, however, less reliably determined than are changes in hydrogen-ion concentration and proteolysis.

The authors are pleased to acknowledge the help of the N.S.W. Milk Board, particularly in providing a research studentship for one of us (K.O.D.). They are indebted to Messrs R. F. Monroe and W. H. Defty of the Board's supervisory staff for the provision of samples, and to Mrs J. Plunkett for considerable invaluable assistance with the technical work.

REFERENCES

(1) AMERICAN PUBLIC HEALTH ASSOCIATION (1948). *Standard Methods for the Examination of Dairy Products*, 9th ed.
(2) FOLTZ, V. D. & BUSHNELL, L. D. (1940). *J. Dairy Sci.* **23**, 973.
(3) NELSON, F. E. (1940). *J. Bact.* **39**, 263.
(4) LIND, H. E. (1940). *J. Milk Tech.* **3**, 208.
(5) FOLTZ, V. D. & NELSON, F. E. (1939). *J. Dairy Sci.*, **22**, 422.
(6) NELSON, F. E. (1943). *J. Bact.* **45**, 395.
(7) WILSON, G. S. (1936). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 206.

(*MS. received for publication* 18 *May* 1954)

637.128:576.809.518

571. THE EFFECT OF MENADIONE (2-METHYL-1:4-NAPHTHOQUINONE) ON THE KEEPING QUALITY OF MILK

By CONSTANCE HIGGINBOTTOM

The Hannah Dairy Research Institute, Kirkhill, Ayr

(With 6 Figures)

In a paper originally presented to the American Chemical Society in September 1953, Kelley & Dittmer (1) reported that the addition of menadione (2-methyl-1:4-naphthoquinone) in a concentration of 0·1 μg./ml. markedly retarded the souring of milk held at 37° C. Milk which was sour in 12 hr. did not become sour in the presence of 0·1 μg. of menadione until after incubation for 23–24 hr. at 37° C. Further, Kelley & Dittmer claimed that the milk produced by cows fed 25 mg. menadione per cow per day had a better keeping quality than the milk from untreated cows. However, in one experiment they found no difference in the rate of souring of the milk from treated and untreated cows.

In the present investigation the effect of the addition of menadione directly to milk on the keeping quality and the bacterial flora of milk held at 37° C. has been studied, and the keeping quality at 37, 22 and 15·5° C. of the milk from cows given a daily dose of 100 mg. of menadione per cow has been determined.

METHODS

In vitro *experiments*

Because of its low solubility in water the required amount of menadione was added to sterile tubes (or flasks) as 0·5 or 1 ml. of an alcoholic solution. The alcohol was allowed to evaporate or was removed by evacuation. Aliquots of the bulked milk from individual herds were added to flasks containing menadione and incubated at 37° C. The milks were examined at intervals or after a given interval for clot formation at 37° C. or for clot-on-boiling. The rate of acid production in the milk was determined by titration with N/9-NaOH and the pH measured electrometrically.

Changes in the bacterial flora were observed in Breed smears of the milk and by the use of selective media. The total viable account was determined on 'Yeastrel'-milk agar, and the Gram-positive flora on glucose agar containing 0·05 % thallous acetate as recommended by Sharpe (2). Enterococci were estimated on the azide medium of Hajna & Perry (3) after incubation at 45° C. Coliform bacteria were counted by the dilution method in McConkey broth (presumptive coliform test) or by colony count on McConkey agar.

For the examination of pure cultures of bacteria 10 ml. broth (Lemco-peptone or Yeastrel-glucose-peptone) or litmus milk were added aseptically to tubes containing the required amount of menadione. After warming them to 37° C. and shaking them to dissolve the menadione the tubes were inoculated with one drop of a 24 hr. culture (or a suitable dilution) and incubated at 37° C. To test for bacteriostasis in tubes showing no change in litmus milk or no turbidity in broth, subcultures were made to sterile media containing no menadione and examined for growth on incubation at 37° C.

In vivo *experiments*

Two groups (A and B) of four Ayrshire cows of the Institute herd were under observation for 50 days from January to March 1954. These cows were all at about mid-lactation, i.e. 104th to 146th day in milk. They were fed dried grass and grass silage for maintenance and 1 gal. milk and concentrates for production over 1 gal. The daily ration of concentrates varied from 8 to 12 lb. For the first 40 days the basic ration consisted of 25 lb. silage plus 14 lb. dried grass, and for the following 10 days it consisted of the same basic ration plus 9 lb. dried grass and 10 lb. fodder beet. The milk yield per day varied from 15 to 30 lb. and averaged 22 lb.

No treatment was given for the first 5 days of the observation period. In period 1 (5th to 25th day) the four cows in group B were each given 100 mg. of menadione per day, while those in group A were not dosed. In period 2 (26th to 48th day) cows in group B were not treated, but those in group A received 100 mg. menadione per day. Each dose consisted of 100 mg. menadione mixed with approximately 8 g. brown sugar and was wrapped in tissue paper. The packages were placed directly into the mouth of the cows in the treated group.

Milk samples. These were obtained at the evening milking at intervals throughout the 50-day period. The eight cows were milked before the rest of the herd and the bulked milk from each group kept separate. The normal routine of machine-milking and of cooling by water using a surface cooler was maintained to obtain a normal microflora in the milk. The milk from the control group was cooled first and was followed by that from the treated group. Samples were taken from the two cans containing the cooled milk from each group.

Milk from individual cows was sampled directly from the milking-machine bucket and cooled in the laboratory.

Examination of the milk. The bulked milk from each group was divided into three portions which were immediately incubated at 37, 22 and 15·5° C. respectively. The milks were examined at intervals for titratable acidity and for instability to 68% alcohol and to boiling. The keeping quality was assessed as the time required to reach (*a*) a titratable acidity equivalent to 2·0 ml. N/9-NaOH, (*b*) instability to 68% alcohol and (*c*) instability to boiling. The microflora of the milk was examined at intervals in Breed smears.

RESULTS

In vitro *experiments*

Milk. Three raw milks of low bacterial count were held at 37° C. in the presence of concentrations of menadione from 0 to 1000 μg./ml. and examined after 24 hr. (Table 1). Clotting at 37° C. was not delayed by 10 μg. menadione/ml., and only one milk showed a marked retardation of acid production, as measured by pH, at this concentration of menadione. The time required to reach the clot-on-boiling stage was not affected by 100 μg. and in one milk not by 1000 μg. menadione/ml. Examination of Breed smears showed that while the microflora in the absence of menadione consisted of pairs of cocci, with increasing concentrations of menadione the streptococcal flora was gradually replaced by short rods, and in the presence of 1000 μg. menadione/ml. consisted almost entirely of rods.

In a sample of pasteurized milk with a presumptive coliform test positive in 1 ml. the titratable acidity after 24 hr. at 37° C. was unaffected by 1000 μg. menadione per ml., presumably due to the growth of the relatively insensitive coliform bacteria.

Table 1. *The effect of the concentration of menadione on the* pH *and the clotting of milks incubated at* 37° C. *for* 24 *hr.*

	Initial quality of milk				Menadione (μg./ml.)					
Milk	Plate count per ml.	P.C.T.	pH	Test	1000	100	10	1	0·1	0
1	2300	Negative in 1 ml.	6·7	pH	6·4	5·9	5·55	4·65	4·6	4·7
				Clot (37°)	−	−	+	+	+	+
				C.O.B.	−	+	+	+	+	+
2	4700	Positive in 0·1 ml.	6·6	pH	5·6	5·4	4·85	4·8	4·75	4·8
				Clot (37°)	−	−	+	+	+	+
				C.O.B.	+	+	+	+	+	+
3	4400	Negative in 1 ml.	6·65	pH	6·6	5·7	4·85	4·75	4·7	4·7
				Clot (37°)	−	−	+	+	+	+
				C.O.B.	−	+	+	+	+	+

P.C.T., presumptive coliform test. C.O.B., clot-on-boiling.

Examination of a fourth raw milk showed that 10 μg. menadione per ml. had no effect on the time to clot-on-boiling and little effect on the titratable acidity or on the numbers of Gram-positive bacteria at the time of clot-on-boiling (14 hr. at 37° C.). There was some indication that not only were the coliform bacteria and enterococci not inhibited but that these bacteria were actually stimulated by the presence of 10 μg. menadione/ml.

The effect of menadione varied with individual milks. In Fig. 1 the development of titratable acidity and the onset of instability to boiling (clot-on-boiling) is given for three milks which showed widely varying responses to the presence of menadione. Milk 7 was unaffected by 1000 μg. menadione/ml., although a slight retardation of acid production was shown after 16 hr., but the milk clotted on boiling after 14 hr. at 37° C. irrespective of the concentration of menadione. Milk 8, on the other hand, showed complete inhibition of acid production by 100 μg. menadione/ml., but only a slight delay in acid production with 10 μg. menadione/ml. milk. Milk 5 showed an intermediate degree of sensitivity to menadione. Further examination of these milks showed that these differences in sensitivity were related to the bacterial flora. Milk 7 had an initial count (log) of 3·64 and contained coliform bacteria in 0·1 ml. milk (log count 1·15). These coliform bacteria grew at all concentrations of menadione. After 12 hr at 37° C. the Gram-positive flora showed some inhibition of growth with 100 μg. menadione, but the total count was only affected by 1000 μg. menadione (Fig. 2). Coliform bacteria were not detected in milk 8 even after incubation. The total and Gram-positive flora were not affected by 10 μg. menadione but were inhibited by 100 μg. and markedly so by 100 μg. menadione/ml. milk. The flora consisted mainly of a streptococcus resembling *Streptococcus agalactiae* and showing in pure culture a similar sensitivity to menadione (Table 2). Enterococci were present in small numbers and showed some stimulation of growth in the presence of 10 and 100 μg. menadione compared with that in the control milk. Milk 5 contained a more mixed flora of streptococci, micrococci and coliform bacteria, and rods predominated in the smears from this milk after incubation with 1000 μg. menadione/ml.

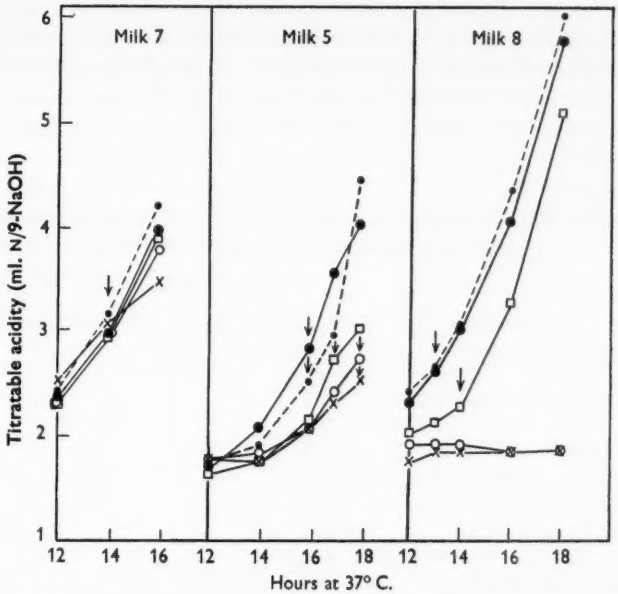

Fig. 1. The effect of the direct addition of menadione on acid production in milks held at 37° C. Menadione (μg./ml.); •---•, 0; ●——●, 1; □——□, 10; ○——○, 100; ×——×, 1000. Arrow: time when milk clotted on boiling.

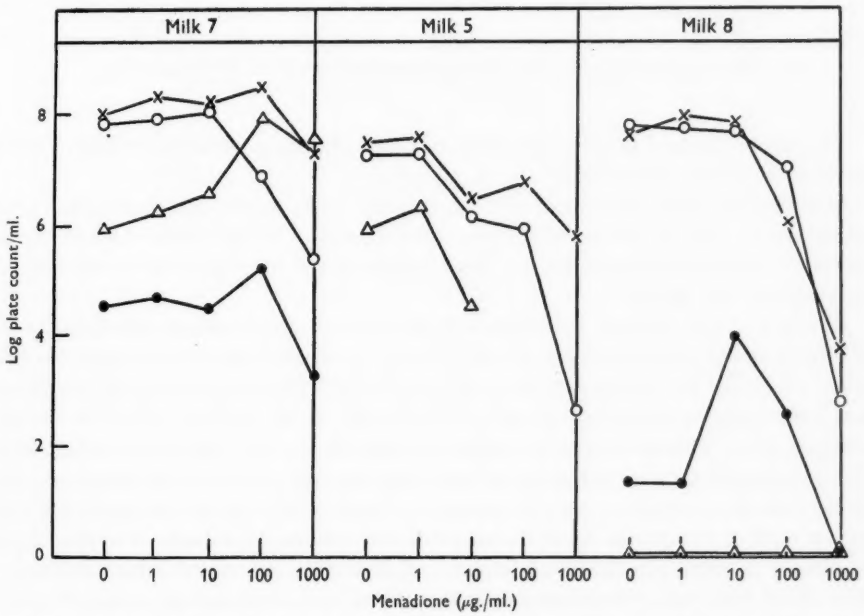

Fig. 2. The effect of the direct addition of menadione on the bacterial count of milks after incubation at 37° C. for 12 hr. ×, total count; ○, Gram-positive flora; ●, enterococci; △, coliform bacteria.

It is evident that the effect of menadione on the keeping quality of milk depends on the microflora of the milk, and that it will be least where the numbers of coliforms are large compared with the streptococci and the micrococci. All the milks examined had low initial counts, the highest being 16,000 bacteria/ml. A more marked effect of menadione on the keeping quality might be expected in milks with a high count, especially where coliform bacteria were absent.

Table 2. *The effect of menadione on the growth of individual species of bacteria in broth and litmus milk after* 24 *hr. at* 37° C.

		Menadione (μg./ml.)					
Bacterial species	Medium	0	1	10	100	1000	2000
Micrococcus (3 strains)	B	+	+	+	(−)	−	
	L.M.	+	+	+	(−)	(−)	
Staph. aureus (coagulase-positive)	B	+	+	+	−	−	
	L.M.	+	+	(−)	−	−	
Str. agalactiae	G.B.	+	+	+	−	−	
	L.M.	+	+	(−)	−	−	
Str. lactis (2 strains)	G.B.	+	+	+	(−)	−	
	L.M.	+	+	+	(−)	(−)	
Str. faecalis (3 strains)	G.B.	+	+	+	+	(−)	(−)
	L.M.	+	+	+	+	(−)	(−)
Bact. coli I (3 strains)	B	+	+	+	+	+	(−)
	L.M.	+	+	+	+	+	(−)
Bact. aerogenes I	B	+	+	+	+	+	
	L.M.	+	+	+	+	+	
Gram-negative rod (1) (3 strains)	B	+	+	+	+	+	
	L.M.	+	+	+	+	(−)	
Gram-negative rod (2)	B	+	+	+	(−)	(−)	
	L.M.	+	+	+	(−)	(−)	

B, Lemco-peptone broth; G.B. glucose-Yeastrel-peptone broth; L.M. litmus milk.
+, growth. −, no growth. (−), growth on subculture.

The results observed in the examination of milks containing menadione were confirmed using pure cultures of bacteria.

Individual bacteria. The sensitivity to menadione of several strains of bacteria was estimated in broth or litmus milk. These included strains isolated from the milk during the *in vivo* experiments and strains of *Strep. agalactiae* and *Staphylococcus aureus* recently isolated from the udder.

One drop of a 24 hr. broth culture was added to 10 ml. of the medium and the incidence of growth noted after incubation for 48 hr. at 37° C. Subcultures to fresh medium were made from tubes showing no growth to determine whether the concentration of menadione was bactericidal or merely bacteriostatic. The results, shown in Table 2, confirm the conclusions drawn from the study of the effect of menadione on milk. *Str. faecalis* and most of the Gram-negative rods, including coliform bacteria, were much less sensitive to menadione than other streptococci and micrococci. These results are in agreement with the earlier work of Armstrong, Spink & Kahnke (4) and of Page & Robinson (5) on the relative sensitivity to menadione of *Staph. aureus* and *Bacterium coli* and of Atkins & Ward (6), who found that some streptococci of the viridans and enterococcus groups were less susceptible to menadione than other Gram-positive bacteria.

In vivo *experiments*

The feeding of 100 mg. menadione per cow per day over a period of 3 weeks had no apparent effect on the appetite or the milk yield of the cows.

The keeping quality of the bulked milk from each group of four cows was determined at intervals over a period of 50 days. Three samples were taken in the preliminary period and fourteen in period 1 (21 days), when the four cows in group B were each given 100 mg. menadione/day and the cows in group A served as controls. In period 2 (23 days) the treatment was reversed, group A but not group B being given menadione. Eleven samples of milk were examined in period 2.

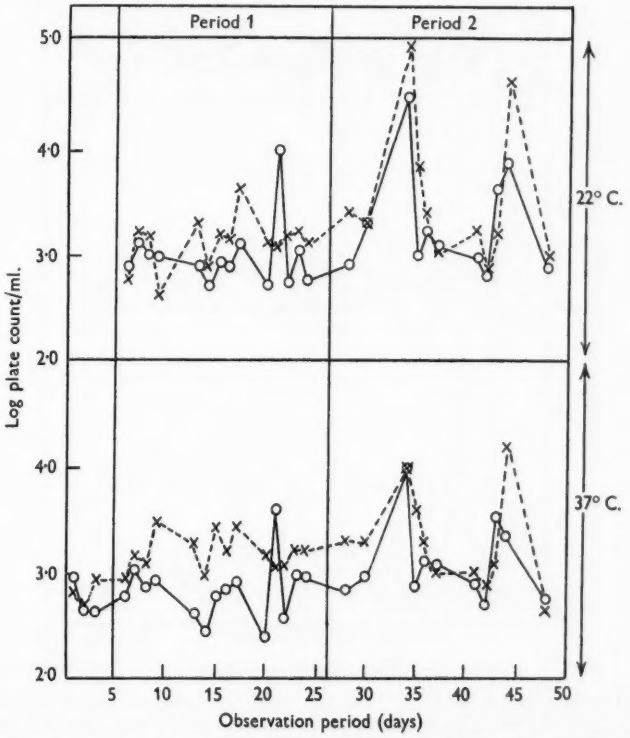

Fig. 3. The log of the plate count on Yeastrel-milk-agar at 37 (3 days) and 22° C. (5 days) of the bulked milk from each group of four cows. Menadione, 100 mg./cow/day, was fed in period 1 to cows in group B and in period 2 to cows in group A. Group A, solid line; group B, broken line.

The bacterial quality of the milk was determined after holding at 15·5° C. for 18 hr. The plate count at 37 and 22° C. (Fig. 3) showed no evidence of a variation in the count attributable to the feeding of menadione. All but one sample in group A and two in group B gave a negative presumptive coliform test in 1 ml. milk.

The methylene-blue reduction time at 37° C. was greater than 7 hr. and less than 24 hr. A definite end-point was recorded for the milk samples on 4 days in period 1. The milk from group A had the longer reduction time on 2 days and group B on the other 2 days. The methylene-blue reduction time at 15·5° C. was recorded for milks obtained on

21 days. On 17 days milks from cows in group B had a reduction time of ½–1 day longer than those from group A, irrespective of whether the milks were obtained in periods 1 or 2. The milks from cows of groups A and B had the same reduction time on the other 4 days. No indication was therefore obtained of any effect on the reduction time at 37 or 15·5° C. such as was obtained by Kelley & Dittmer (1) with milks from cows dosed with menadione.

Keeping quality of the milk. The keeping quality of the milk held at 37° C., when assessed by the time required to develop a titratable acidity equivalent to 2 ml. N/9-NaOH, to become unstable to 68% alcohol or to clot-on-boiling is shown in Fig. 4. The differences

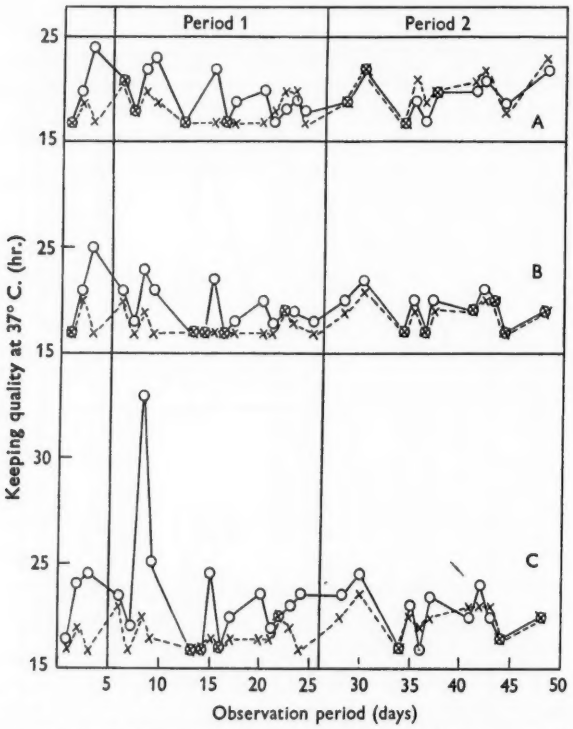

Fig. 4. The keeping quality of milk held at 37°C. assessed as the time required to reach (A) a titratable acidity equivalent to 2 ml. N/9-NaOH, (B) instability to 68% alcohol and (C) instability to boiling. Menadione, 100 mg./cow/day, fed in period 1 to cows in group B and in period 2 to cows in group A. Group A, solid line; group B, broken line.

in the keeping quality of the milks from groups A and B were small. Milk from cows in group A usually remained stable to alcohol or to boiling for 1–2 hr. longer than that from cows in group B, irrespective of which group was receiving menadione.

The corresponding results for the same milks when held at 22 or 15·5° C. are shown in Figs. 5 and 6. For these milks the end-points are given for the 12 hr. intervals after the first interval of 18 hr. Here too there was no regular difference between the milks from the two groups of cows. Even where the end-point was recorded as differing by one 12 hr. period, the actual difference in keeping quality was frequently only a matter of 6 hr. or less.

Milk from individual cows was examined on the 3rd day (preliminary period) and on the 35th and 36th days and 43rd and 44th days (period 2). There was no consistent difference between the keeping quality at 37, 22 or 15·5° C. of the milk from group A and that from group B. When the very small numbers of the natural flora were augmented by the addition to 250 ml. milk of one drop of a 24 hr. culture of *Str. agalactiae*, a coagulase-positive *Staph. aureus* or *Str. faecalis*, the keeping quality of the milks showed no differences attributable to the treatment of the cows.

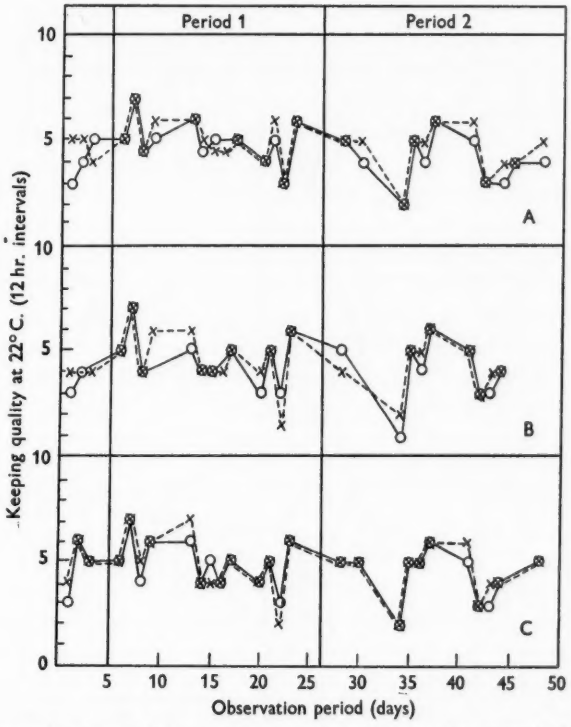

Fig. 5. The keeping quality of milk held at 22° C. assessed as the time taken to reach (A) a titratable acidity equivalent to 2 ml. N/9-NaOH, (B) instability to 68% alcohol and (C) instability to boiling. The time of incubation is shown on the ordinates as 0–10 units: 1, 0–18 hr.; 2, 19–30 hr. and each subsequent unit represents a 12 hr. interval up to 10, 115–126 hr. Menadione, 100 mg./cow/day, fed in period 1 to cows in group B and in period 2 to cows in group A. Group A, solid line; group B, broken line.

Microflora of the milk. An examination of the morphology of the microflora was made at intervals throughout the incubation of all the milks. The predominating flora at the end of the keeping quality period was isolated from the milks held at the three temperatures on 8 days in period 1. In addition, at the end of the period of examination a loopful of milk was transferred to McConkey broth and to azide medium to determine whether or not coliforms and enterococci were present.

Examination of the flora showed that in general the morphological types were similar in milks obtained on the same day and incubated at the same temperature irrespective of whether the milk was obtained from cows in group A or in group B. Differences in the

relative proportions of the morphological types did occur, but only in a few instances was the flora very markedly different, as when spore-forming bacilli overgrew the other species in milk from one group of cows but not in that from the other group. At 37° C. streptococci and micrococci predominated and rods were few in number, except in a few milks where spore-forming bacilli or *Bact. coli* overgrew the coccal flora. At 22° C. short rods predominated in most milks, but cocci were always present and in a few milks micrococci were the predominant morphological type. At 15·5° C. short rods always predominated and there were usually few cocci. These short rods were mainly Gram-negative species

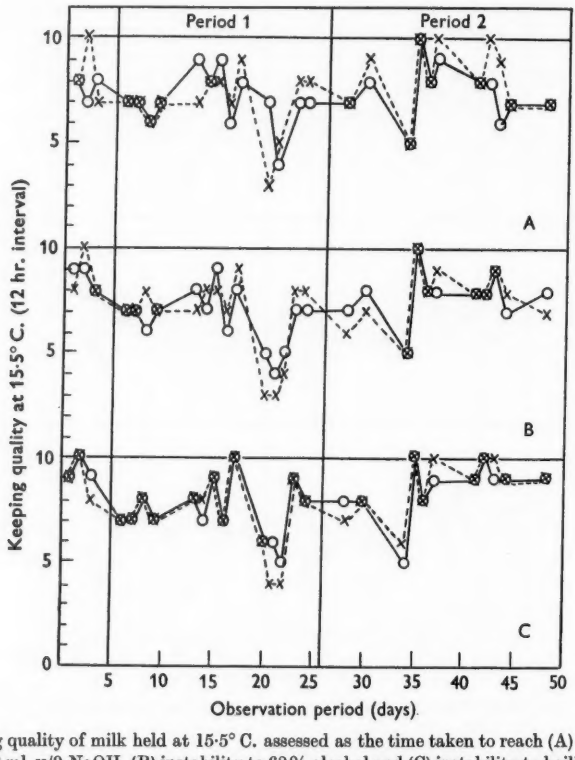

Fig. 6. The keeping quality of milk held at 15·5° C. assessed as the time taken to reach (A) a titratable acidity equivalent to 2 ml. N/9-NaOH, (B) instability to 68% alcohol and (C) instability to boiling. Time of incubation is shown on the ordinates as 0–10 units: 1, 0–18 hr.; 2, 19–30 hr., and each subsequent unit represents a 12 hr. interval up to 10, 115–126 hr. Menadione, 100 mg./cow/day, fed in period 1 to cows in group B and in period 2 to cows in group A. Group A, solid line; Group B, broken line.

producing alkali or inert in litmus milk, but included *Bact. coli* and *Bact. aerogenes*. The sensitivity of pure cultures of representative strains of the micrococci and the Gram-negative rods in milks held at 22 and 15·5° C. was examined. As shown in Table 2 the Gram-negative rods were relatively insensitive to menadione, although a few strains and the micrococci were inhibited but not killed by 100 μg. menadione/ml.

Enterococci (*Str. facealis*) were detected in all the milks examined after incubation at 15·5 and 22° C. and in milks from group A, but not always from group B after incubation at 37° C. The frequent occurrence of *Str. faecalis* in milk from group A was traced to the

presence of a reservoir of these bacteria in one-quarter of the udder of one of the cows in group A.

Bact. coli, type I, or *Bact. aerogenes*, type I, was detected in a loopful of milk in about one-third of the milks after incubation at 37° C. and in approximately two-thirds of the milks after incubation at 22 or 15·5° C., although coliform bacteria were absent in 1 ml. of the fresh milks (with three exceptions). The detection of *Str. faecalis*, *Bact. coli* or *Bact. aerogenes* in the milk did not necessarily indicate the predominance of these bacteria in the milk.

Menadione in the milk. Immediately after sampling, milk from treated and from untreated cows was placed in a deep-freeze cabinet until examined. After thawing and centrifuging the cream was extracted with *n*-hexane or treated with alcohol and then extracted with light petroleum (B.P. 40–60° C.). The extract was dried over anhydrous sodium sulphate, and the solvent removed by vacuum distillation. The residue was tested for menadione or related quinones by the method of Craven (7), using ethyl cyanoacetate, and by that of Novelli (8), using 2:4-dinitrophenylhydrazine. The concentrated extracts from 200 ml. milk from either the treated or the control cows failed to show any reaction with either method. Pinder & Singer (9) obtained a blue colour with the ethyl cyanoacetate method with as little as 0·1 mg. menadione/ml., and this limiting sensitivity was confirmed. Hence the amount of menadione extracted from 200 ml. of the milks was less than 0·1 mg., i.e. less than 0·5 μg. menadione/ml. milk. The addition of as little as 1 μg. menadione/ml. to milk could be detected in the concentrated hexane extract from 200 ml. but not from 100 ml. milk. It was therefore concluded that if present in the milk of the treated cows the concentration of menadione or of related substances giving a blue colour with ethyl cyanoacetate was less than 1 μg./ml. milk. With cows receiving 100 mg. menadione/day and giving 20 lb. milk/day, only about one-tenth of the dose would have to pass into the milk to give 1 μg./ml.

DISCUSSION

The keeping quality of the milk from eight cows was unaffected by the feeding of menadione (2-methyl-1:4-naphthoquinone) at a level of 100 mg. menadione/day. This is not in agreement with the work of Kelley & Dittmer (1), who reported that the milk from cows given 25 mg. menadione/day kept sweet at 37° C. for 6–12 hr. longer than milk from control cows or from the same cows before and after treatment. In one experiment Kelley & Dittmer failed to obtain any difference in the rate of souring of milk from treated and untreated cows, although when the experiment was repeated after a lapse of 2 months the milk from the menadione-treated cows did show a longer keeping quality than that of the controls. Kelley & Dittmer pointed out that the diet of the cows was not the same on the two occasions and also suggested that the bacterial flora may have affected the results, but this point was not investigated.

The greater sensitivity to menadione of Gram-positive bacteria (with the exception of the enterococci and some streptococci of the viridans group) compared with Gram-negative bacteria has been reported (4, 5, 6) and confirmed in the present investigation. It was to be expected therefore that the effect of the direct addition of menadione to milk would vary with the bacterial flora of the milk. Even with the milks of low bacterial content which were examined in the present investigation a concentration of 10 μg. menadione/ml. of milk had little effect on the souring of the milk. Kelley & Dittmer, however, found an

appreciable delay in the souring of milk to which was added only 0·1 μg. menadione/ml. milk. This suggests that the microflora of the milks examined by Kelley & Dittmer consisted of species more sensitive to the action of menadione than those examined in the present investigation.

In this work the microflora of the milks from the treated and untreated cows did not differ appreciably. In milks incubated at 37° C. the microflora consisted mainly of Gram-positive cocci, and for this reason might have been expected to show some response to the treatment of the cows with menadione. In milks incubated at 22 and 15·5° C., Gram-negative bacteria, including coliform bacteria, were the main types present, and milks held at these temperatures would be less likely to be affected by menadione. Kelley & Dittmer, however, reported a longer keeping quality at 20 or 10° C. for milk from menadione-treated cows than from control cows.

The failure to confirm the results of Kelley & Dittmer might have been due to the use of four times the dose of menadione which was used by the American workers. They did find a falling off in response to 100 mg. menadione in one experiment in which the cows had been given a daily dose of 25 or 50 mg. of menadione on the previous 30 days. A change in the bacterial flora of the milk might also account for this apparent decrease in response to higher doses of menadione. A variation in the keeping quality of the milk from treated and untreated cows cannot be accepted as valid unless it can be shown that it is not due to variation in the numbers and types of the bacterial flora of the milk which might have occurred even had no treatment been given. Most of the bacteria in the milk of healthy cows are derived from contact with milking machines and other dairy equipment, and no two milks are likely to contain exactly the same numbers and species of bacteria.

Tests for the presence of menadione in the milk from treated cows showed that the concentration was less than 1 μg. menadione/ml. This concentration of menadione added to milk had no apparent effect on the bacteria in the milks used in the present investigation. The possibility remains that any effect on the bacterial flora of milk may be due to some substance other than menadione which appears in the milk as a result of feeding menadione. The most likely explanation of the results obtained by Kelley & Dittmer is that differences in the bacterial flora itself rather than the action of some inhibitory substance on the bacteria was responsible for the differences which they observed in the keeping quality of the milk from treated and control cows.

SUMMARY

1. The addition of 1 μg. menadione/ml. to milk had no effect on the souring of the milk at 37° C. With higher concentrations of menadione the effect on the keeping quality at 37° C. varied with different milks. In some milks the clot-on-boiling stage was delayed by 1 hr. with 10 μg. menadione and by 2 to over 5 hr. with different milks containing 100 μg. menadione per ml. Some milks, however, showed no alteration in the keeping quality at 37° C. with as much as 1000 μg. menadione/ml.

2. The response of individual milks to the presence of menadione appeared to be related largely to the bacterial flora of the milk. The relative insensitivity of Gram-negative rods, including coliform bacteria and of enterococci, to menadione was confirmed. Milks containing large numbers of coliform bacteria showed the least and those free of such bacteria the greatest response to menadione.

3. Menadione fed to milking cows at the rate of 100 mg./day for a period of 3 weeks had no effect on the keeping quality of the milk when it was held at 37, 22 or 15·5° C. The time of incubation required for the milk to attain a titratable acidity equivalent to 2 ml. N/9-NaOH, to become unstable to 68% alcohol or to boiling showed no difference between the milk from the cows given menadione and that from the control cows.

4. The menadione had no demonstrable effect on the appetite or the well-being of the cows nor on their milk yield.

5. When as much as 100 μg. menadione was given per cow per day, no menadione was detected in the milk under conditions suitable for the detection of 1 μg. menadione/ml. milk.

REFERENCES

(1) KELLEY, G. G. & DITTMER, K. (1954). *Milk Dlr.* **43**, 105.
(2) SHARPE, M. E. (1952). *J. Hyg., Camb.* **50**, 210.
(3) HAJNA, A. A. & PERRY, C. A. (1943). *Amer. J. publ. Hlth*, **33**, 550.
(4) ARMSTRONG, W. D., SPINK, W. W. & KAHNKE, J. (1943). *Proc. Soc. exp. Biol., N.Y.*, **53**, 230.
(5) PAGE, J. E. & ROBINSON, F. A. (1943). *Brit. J. exp. Path.* **24**, 89.
(6) ATKINS, P & WARD, J. L. (1945). *Brit. J. exp. Path.* **26**, 120.
(7) CRAVEN, R. (1931). *J. chem. Soc.* p. 1605.
(8) NOVELLI, A. (1941). *Science*, **93**, 358.
(9) PINDER, J. L. & SINGER, J. H. (1940). *Analyst*, **65**, 7.

(*MS. received for publication* 5 *July* 1954)

637.127:541.18.04

572. THE HEAT COAGULATION OF MILK

By G. T. PYNE and KATHLEEN A. McHENRY
Dairy Chemistry Department, University College, Cork

(With 1 Figure)

Despite numerous investigations the factors concerned in the heat coagulation of milk remain in great part obscure. Casein is known to be the constituent which undergoes coagulation; salt unbalance, particularly if it takes the form of excess calcium and magnesium, is thought to be the main contributory factor (1). But heat coagulation cannot in general be correlated with milk or milk-salt composition (2); anti-coagulative treatments, such as preheating and addition of certain anions, are largely empirical in development and application. A general theory of the phenomenon is lacking.

Heat and rennet coagulations, despite obvious differences, have certain basic similarities. Both involve types of casein denaturation which render the protein more sensitive to the precipitating action of calcium ions. This similarity suggests that factors which are of special importance for the second or precipitation stage of rennet action may also be important for the corresponding stage of heat coagulation and may play a determining role in it. These factors are primarily calcium-ion concentration itself and secondarily colloidal phosphate content. Association of colloidal phosphate with caseinate has recently been shown by one of the present writers (3) to increase markedly the sensitivity of caseinate, when renneted, to calcium ions. It seemed worth while to investigate how far calcium-ion concentration and colloidal phosphate content might influence the heat coagulation of milk. In the course of the work, other, mainly related factors, emerged, and the scope of the investigation necessarily widened. This paper presents an account of the results obtained.

METHODS

(*a*) Experiments were conducted chiefly on separated milk from individual Dairy Shorthorn cows, mainly in mid-lactation, also on bulked separated milk and on reconstituted spray-dried skim milk, and in a few instances on solutions of calcium caseinate and caseinate-phosphate complexes as prepared by Powell & Palmer (4). Between experiments samples were held at 0–2° C.

(*b*) Lactose-free milk was prepared by dialysis of a relatively small quantity of spray-dried skim milk dissolved in twice its weight of water against two changes of an artificially prepared lactose-free milk serum. Endosmosis during dialysis was found to bring this milk to approximately the concentration of average milk in respects other than lactose.

(*c*) Albumin-poor milk was prepared by 10-fold dilution of the Chamberland filter deposit from skim milk with the corresponding Chamberland serum freed from albumin by 15 min. boiling and filtration. Normal milk for comparative purposes was similarly prepared using unboiled instead of boiled serum. Both reconstituted milks were then brought to exactly the same salt content by dialysis against a relatively large volume of the original unfiltered separated milk.

(*d*) Artificial milk serum usually contained about 8·3 mM/l. sodium citrate, 11·7 mM/l. sodium phosphate, 8·3 mM/l. calcium chloride, 2·5 mM/l. magnesium chloride, 25·0 mM/l. sodium chloride and 5% hydrated lactose, and its pH was 6·6. Citrate was added as citric acid and phosphate as the primary salt. Other salts were added as chlorides, and after the mixture had been diluted to nearly its final volume, the pH was adjusted to 6·6 by careful addition, with rapid mechanical stirring, of 0·5 N-NaOH.

(*e*) Coagulation times were usually determined by visual observation of the samples contained in narrow sealed Pyrex tubes and immersed in a glycerol bath which was maintained at 130° C. and provided with a rocking device on the lines of that used by Cole & Tarassuk(5). The temperature 130° C. was chosen as allowing conveniently rapid coagulations without much risk of altering the relative coagulation order of samples observed at lower temperatures(5). Coagulation times at temperatures between 100 and 130° C., if fairly short, were determined similarly. If relatively long and where great accuracy was not required, they were usually determined approximately by visual observation at 15 min. intervals, the samples being heated in a pressure cooker. Samples were placed in small flasks or test-tubes fitted with capillaries to allow equalization of pressure. Evaporation losses, slight under these conditions, were always made good when the containing vessels were removed for observation or analysis.

(*f*) pH was determined by glass electrode and titratable acidity with alkalis in the usual way. Sometimes, however, titratable acidity was measured after addition of 4% of a saturated solution of neutral potassium oxalate if it was desired to eliminate the contribution of heat-induced displacements of calcium phosphate equilibrium to the acidity (cf. Kaloyereas(6)). Protein content was determined by formaldehyde titration, colloidal phosphate as the difference between trichloracetic acid soluble and dialysable phosphate. Phosphate was estimated in the trichloracetic filtrate or in the dialysate by the Fiske & Subbarow(7) colorimetric method. As these sera usually contained sufficient nitrogenous matter to give turbid solutions with the molybdate reagent, an intermediate separation of the phosphate by adding magnesia mixture, standing overnight, centrifuging, decanting and rejecting the supernatant liquid was practised. Dialysis, unless otherwise specified, was always conducted for 48 hr. at 0–2° C.

(*g*) *Effective calcium-ion concentration.* The term 'effective' is used here to indicate that the calcium-ion concentrations obtained as described below are composite values, related, no doubt, to the true calcium-ion concentrations but also influenced to some degree by the presence of certain other ions, magnesium for instance, and by sample-to-sample variations in citrate and phosphate content. These values provide, however, a useful practical measure of the relative coagulative powers of the divalent milk cations of the various milks as modified by the anions present. The 'effective' calcium-ion concentration of milk samples was determined by a renneting technique based on that already described(8). A small quantity of good-quality reconstituted spray-dried skim milk was brought to the calcium-ion concentration of the sample under investigation by dialysis against a considerable excess of the latter, and then treated with sufficient dilute rennet solution (previously dialysed free from salts) at 0–5° C. to ensure complete conversion of caseinate to paracaseinate in an hour or less. The coagulation time of the paracaseinate at some definite suitable temperature, e.g. 35° C. or, alternatively and more conveniently, the temperature at which its coagulation occurred in some definite suitable time, between say 60 and 120 sec., was determined. The times or temperatures thus obtained were

expressed in terms of the calculated equivalent calcium-ion concentrations (or 'effective' calcium-ion concentrations) by the use of a calibration curve prepared by plotting the observed renneting times (or temperatures) against calcium-ion concentrations for a similar dried milk solution equilibrated against artificial milk sera of varying calcium-ion concentration and constant, approximately average, milk concentrations of phosphate, citrate and other anions. Calcium-ion concentrations of calcium salt solutions containing citrate were calculated from the results of Hastings, McLean, Eichelberger, Hall & da Costa (9). It should be noted that the 'effective' calcium-ion concentrations obtained (cf. Fig. 1) were higher than the calcium-ion concentrations found by Smeets & Seekles (10) who used a direct chemical method. They obtained values of 2·0–3·6 mM./l. of milk *ultrafiltrate*.

RESULTS

(1) *Condensed individual milks (summary of preliminary experiment)*

Ten individual skim milks were each divided into two parts, one part being stabilized by heating at 120° C. for 7 min. All samples were then condensed to 22% milk solids. Great differences in the heat coagulability of these milks were observed over the range 100–120° C., the values varying from 1·5 min. at 100° C. to 20 min. at 120° C. Coagulation times showed no obvious correlation with analytical results other than those for the effective calcium-ion concentration of the milks in condensed form, and, to a lesser degree, their effective calcium-ion concentration prior to condensation. Preheating reduced effective calcium-ion concentration in all cases, and usually, but not invariably, improved heat stability.

In view of these preliminary results it was decided to concentrate on the apparent relation between coagulability and calcium-ion concentration. Natural or artificial uncondensed milks, however, were used in this study from now on instead of the more complex condensed product, since the changes in pH, in salt equilibrium and in serum protein content induced by condensation seemed likely to obscure the relation sought.

(2) *Influence of calcium-ion concentration and heat-induced acidity on coagulation*

Fig. 1 (upper curve) shows coagulation times and effective calcium-ion concentration for twenty-six individual milk samples. The two appear on the whole to be inversely related. Correlation of coagulation time with other analytical milk results (not recorded here) was not immediately evident, but the existence of a correlation with developed acidity emerged on closer examination. All samples were noticed to have become slightly acid on coagulation—with pH values from about 5·5 to 6·0—those which coagulated fastest being in general the least acid, and those which coagulated slowest the most acid. The degree of acidity which developed in a sample during coagulation, as represented by its pH shift, was, in fact, roughly proportional to its coagulation time (Fig. 1, lower curve), and was thus inversely related to its initial calcium-ion concentration. These findings suggested that coagulation of casein might arise through the action of initial calcium-ion concentration supplemented by that of heat-developed acidity, an acidity originating presumably in thermal decomposition of lactose and being thus approximately proportional in amount to the duration of the heating process. A reciprocal relation between coagulation time and initial calcium-ion concentration would, on this view, be natural. In associating developed acidity with calcium-ion concentration as joint coagulating agencies, it is not, of course, intended to convey the impression that these activities are

necessarily independent. Developed acidity may in fact function chiefly as a provider of additional calcium-ions by making soluble previously insoluble calcium combinations, and through increased ionization of calcium citrate and other soluble calcium salts.

Confirmation of the importance of the serum constituents—and thus indirectly of the importance of calcium ions—for heat coagulation is furnished by the behaviour of mutually dialysed milk samples. Five individual milks which coagulated at 130° C. in 22, 29, 38, 49

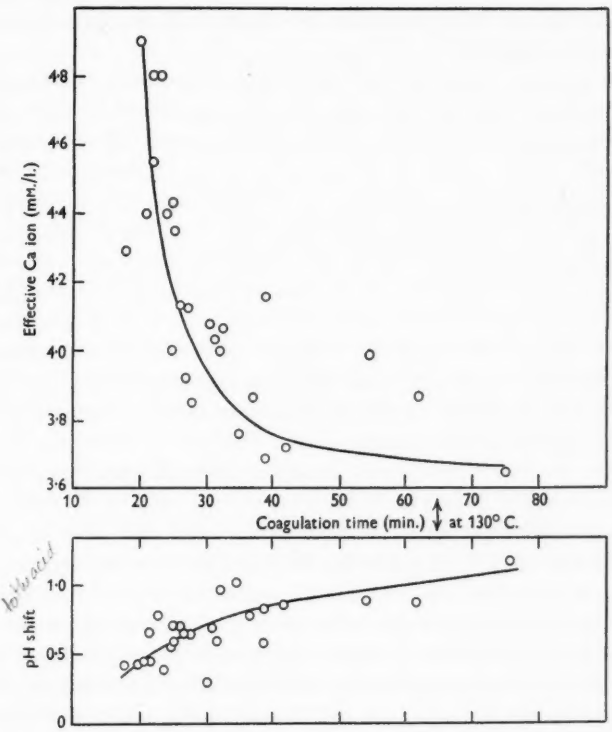

Fig. 1. The relation between effective calcium-ion concentration and coagulation time of milk samples at 130° C. (upper curve). The relation between the pH shift at coagulation and coagulation time of milk samples at 130° C. (lower curve).

and 58 min. respectively were found to coagulate in 38, 38, 38, 36 and 38 min. respectively after all had been dialysed for 48 hr. at 0–2° C. against a relatively large volume of mixed milk composed of equal proportions of each.

The foregoing view of the heat coagulation of milk was tentatively advanced in a preliminary note (11) based on less complete data than those now available. The question was raised (12), however, whether development of acidity during heating, instead of being a direct factor in coagulation, might not be merely an inevitable and more or less proportional consequence of a heating time really determined by other factors. This point will now be examined.

(3) *Lactose-derived acidity as a factor in heat coagulation*

Formation of acid through thermal decomposition of lactose has long been known to occur in heated milk. The reaction is catalysed by certain milk salts, e.g. phosphates and citrates, and especially by caseinate (13). Gould (14) finds most of the acid formed to be formic; some other volatile acids are also present and a little lactic acid. Calculations from his results indicate that about two-thirds of the titratable acidity developed in milk heated for 2 hr. at 116° C. can be ascribed to the production of these acids. In an attempt to determine how far this acid production is a factor in heat coagulation the following experiments were carried out.

(*a*) *Attempted removal of developed acid by dialysis.* Removal of developed acidity by periodic neutralization would appear to be the obvious method of determining its relevance to heat coagulation. In view of possible disturbance of milk-salt equilibrium, however, this procedure was rejected in favour of removal of developed acid by dialysis of the heated sample against considerable excess of the same milk unheated. The results were most unexpected. Milk heated a relatively short time was found to be destabilized by this treatment, whereas milk heated for a relatively long time was stabilized. This phenomenon, apparently due, *inter alia*, to various degrees of transfer of colloidal phosphate from unheated to heated milk, will be dealt with in a later article. For the moment it is sufficient to remark that this form of dialysis obviously provided no answer to the question of the contribution of developed acidity to heat coagulation. An alternative line of investigation was therefore sought in the study of the coagulation behaviour of lactose-enriched and lactose-free milks.

(*b*) *Coagulation of lactose-enriched and lactose-free milks.* The milks used and the results obtained can be indicated briefly as follows. A normal milk of pH 6·68 coagulated in 90 min. at 120° C., pH 5·26, while a sample of the same milk with 5% added lactose coagulated in 60 min. at 120° C., pH 5·30. Lactose-free milk was prepared as described in § (*b*) on p. 60. Lactose-free milk of pH 6·51 coagulated in 90 min. at 120° C., pH 6·15, while a sample of the same lactose-free milk with 5% added lactose coagulated in 60 min. at 120° C., pH 5·92. In both cases presence of lactose or extra lactose increased the rate of production of acid and the coagulability of the sample. The conclusion is unavoidable that acidity, originating partly at least in thermal decomposition of lactose, is *normally* a factor in the heat coagulation of milk. But it is not an *essential* factor since lactose-free milk can both develop acidity and coagulate, though it does so more slowly than milk containing lactose.

(4) *Non-lactose sources of developed acidity*

Two additional sources of acidity besides lactose decomposition exist in milk, one obvious, the other less so. These are displacement of calcium phosphate equilibrium and liberation of phosphate from heat-denatured casein.

(*a*) Heat-induced displacement of milk phosphate equilibrium with precipitation of tricalcium phosphate and shift of reaction towards acidity is well known. The soluble phosphate is the milk constituent primarily involved in the changes; the small amount of colloidal dicalcium phosphate known to occur in milk (15) may also contribute. Acidity from this source is probably liberated early in the heating process (16).

(*b*) Liberation of phosphate from casein-ester combinations is probably a more impor-

tant source of acidity. Howat & Wright (17) observed that calcium caseinate loses about 80% of its ester phosphate and a small proportion of its nitrogen, becoming at the same time acid, during heating for 5 hr. at 120° C. Assuming with Eilers (18) that about one-half of the free acid groups of the casein-ester phosphate are neutralized by calcium at the pH of milk, it is clear that phosphorus cleavage of casein will liberate an approximately equimolecular mixture of primary and secondary phosphates. Interaction of these liberated phosphates with calcium salts can clearly lead to further formation of tricalcium phosphate and development of a corresponding acidity. The fairly rapid fall observed in the P/N ratio of the casein isolated from heated milk after about 60 min. heating at 120° C. suggests that development of acidity in consequence of liberation of phosphate from casein becomes fairly marked in the later stages of heating.

(*c*) An idea of the relative magnitude of the acidities furnished by these various sources can be obtained from the following results for a sample of mixed milk which coagulated in 90 min. at 120° C.:

Milk	pH	Titratable acidity (oxalate) (ml. N/1 per l.)	Total phosphate (mM P/l.)	Soluble phosphate (mM. P/l.)	Colloidal phosphate (mM. P/l.)	P/N ratio for casein
Unheated	6·69	8·4	23·0	11·1	11·9	0·056
Coagulated	5·87	17·2	26·4	9·8	16·6	0·033

Assuming the increase in acid soluble or total phosphate of 3·4 mM P/l. to arise from casein-ester phosphate, and taking one-eighth of the colloidal phosphate present initially to be dicalcic, it can be calculated that nearly one-half of the total developed acidity came from lactose decomposition, one-third from casein-ester phosphate, and the remainder from displacements of equilibria of phosphate initially present.

(5) *Influence of colloidal phosphate on coagulation*

As mentioned in the Introduction colloidal phosphate was expected to be, on the analogy of its effect on rennet coagulation, a factor of importance in the heat coagulation of milk. Experiments on 2·5% solutions of calcium caseinate and calcium caseinate phosphate complex confirmed this expectation, these solutions being found to coagulate equally rapidly (7 min. at 120° C. at pH 6·60 in presence of 15 mM/l. NaCl) at concentrations of 3·25 and 2·5 mM/l. $CaCl_2$ respectively.

Repetition of the experiment with milk yielded similar results. The solutions compared were untreated skim milk and skim milk from which all colloidal phosphate had been removed by addition of 2·5% solid sodium citrate followed by dialysis for 72 hr. against 20 volumes of the untreated skim milk, changed every 24 hr. As the citrated milk was found to have increased somewhat in volume during dialysis the comparison skim milk was diluted to the same degree. Milk serum prepared by dialysis of a relatively small quantity of water against several changes of the same milk was used as the diluent. The colloidal phosphate-free milk, pH 6·70, coagulated in 210 min. at 120° C., pH 5·30, whereas the untreated milk, pH 6·70, coagulated in 120 min. at 120° C., pH 5·61. Presence of colloidal phosphate thus shortened the coagulation time by about 40% and allowed coagulation to occur at a lower acidity.

The experimental results referred to briefly in § 3 (*a*) bear out the importance of colloidal phosphate content for heat coagulation, though the mechanism of the effect there described is not clear.

(6) *Influence of milk albumin on coagulation*

The favourable effect of preheating milk on the heat stability of the evaporated milk prepared therefrom is often attributed in part to the coagulation or denaturation of albumin before condensation has raised its concentration to a dangerous level. Whatever role albumin (or more correctly, serum protein) may play in the heat coagulation of evaporated milk, it does not appear to be important in the coagulation of uncondensed milk.

Thus albumin-poor milk and the corresponding albumin-normal milk prepared as described in § (*c*), p. 60, and both initially at pH 6·70, were found to coagulate in exactly the same time, 120 min. at 120° C., and to possess then exactly the same pH of 5·64.

(7) *Casein denaturation*

The coagulum which forms in heated milk is usually observed to be more jelly-like and tenacious than ordinary acid or rennet curd, to dissolve with greater difficulty in oxalate or citrate solutions, and to furnish with alkalis solutions which are much more viscous than those of casein. Clearly some degree of heat denaturation—a change known to be accompanied, significantly, by reduced base-binding capacity and increased sensitivity to calcium (17)—would seem to have occurred, especially in milks which coagulate slowly, and have been subjected to relatively prolonged heating.

Howat & Wright (17) hesitate to invoke casein denaturation as a cause of coagulation in evaporated milk in view of the complexity of that system and the relative slowness of denaturation. These considerations apply with less force to uncondensed milk, a simpler and more heat-stable system. The increased acid-soluble phosphate content of strongly heated milk and the reduced P/N ratio of the casein obtained therefrom afford evidence that some degree of casein denaturation does in fact occur during the heat coagulation of milk.

DISCUSSION

The foregoing results appear to provide a reasonable basis for a provisional explanation of heat coagulation in milk. It is suggested that calcium-ion concentration and the colloidal phosphate content of the caseinate-phosphate complex of milk are the two main initial factors determining the tendency of a milk to coagulate. Acidity developed during heating (mainly through thermal decomposition of lactose, but partly through phosphate released from denatured casein and to some degree from displacement of phosphate equilibria) and partial denaturation of caseinate (a change accompanied by increased sensitivity of the protein to calcium ions) are the two chief supplementary factors. If the predetermining factors favourable to coagulation, i.e. calcium-ion concentration and colloidal phosphate, are relatively high, only a moderate degree of development of supplementary acidity and casein denaturation will be needed to bring it about. Coagulation will thus be fairly rapid and the coagulum will consist of a relatively slightly altered caseinate-phosphate complex. If, on the other hand, calcium-ion concentration or colloidal phosphate content are relatively low, coagulation will be delayed until the requisite degree of heat-developed acidity and caseinate sensitivity through denaturation is attained to supplement adequately the activity of the primary agencies. Coagulation in these circumstances will be relatively slow, and the coagulum will consist of a relatively more completely denatured caseinate-phosphate complex.

Calcium-ion concentration and the colloidal phosphate content of the caseinate-phosphate complex, though conveniently regarded as separate factors, may not necessarily be independent of each other; it is more probable that they normally form interrelated parts of a single system in equilibrium and tend to vary together. Developed acidity, moreover, as already emphasized, is probably not an independent supplement of calcium-ion activity operating solely in virtue of enhanced hydrogen-ion concentration, but is also, and perhaps chiefly, an indirect source of additional calcium ions released from insoluble or deionizing calcium combinations. Investigation is needed on both these points, however.

The view of heat coagulation advanced here applies only to uncondensed milks. Condensation can obviously introduce such complicating changes of pH, of calcium-ion concentration, salt equilibrium generally and of albumin concentration that milk compositional factors important for the coagulation of the simpler uncondensed system may have much less relevance for the more complex condensed one.

Restricted in scope as they are, some of the conclusions reached may nevertheless bear on certain aspects of evaporated milk practice, for instance, on possible modes of action of certain stabilizing treatments. Stabilizing treatments would appear to operate mainly by effecting a reduction in either calcium-ion concentration or in the colloidal phosphate content of the caseinate phosphate; at least it is difficult to imagine their affecting other coagulation factors such as rate of acid production or rate of casein denaturation. Anion additions thus probably operate either by precipitation or deionization of soluble calcium. Moderate preheating treatments may operate also by calcium precipitation and stronger preheating treatments, perhaps in addition, by detachment of colloidal phosphate from its combination with caseinate (19, 20). The observed replacement of the characteristic polarogram of the barium caseinate-barium phosphate complex by that of barium caseinate following strong heating of the complex (21) is suggestive here.

SUMMARY

1. A study of the compositional factors which affect the heat coagulation of milk has been undertaken.

2. Calcium-ion concentration and colloidal phosphate content appear to be the chief factors determining the tendency of a milk to coagulate on heating.

3. Acidity (mainly derived from thermal decomposition of lactose and casein) and heat denaturation of casein are supplementary coagulation factors which develop during the heating process.

4. Lactose, as the main source of heat-developed acidity, is an important secondary factor in heat coagulation, but not an essential one. Coagulation can proceed, though more slowly, in its absence. The serum proteins play no part in the phenomenon.

5. A provisional theory of the heat coagulation of milk based on these findings is put forward.

REFERENCES

(1) SOMMER, H. H. & HART, E. B. (1919). *J. biol. Chem.* **40**, 571.
(2) ROGERS, L. A., DEYSHER, E. F. & EVANS, F. R. (1921). *J. Dairy Sci.* **4**, 294.
(3) PYNE, G. T. (1953). *Chem. & Ind.* **72**, 302.
(4) POWELL, M. E. & PALMER, L. S. (1935). *J. Dairy Sci.* **18**, 401.
(5) COLE, W. C. & TARASSUK, N. P. (1946). *J. Dairy Sci.* **29**, 421.
(6) KALOYEREAS, S. A. (1954). *Science*, **120**, 111.
(7) FISKE, C. H. & SUBBAROW, Y. (1925). *J. biol. Chem.* **66**, 375.

(8) Pyne, G. T. (1948). *Nature, Lond.*, **162**, 925.
(9) Hastings, A. B., McLean, F. C., Eichelberger, L., Hall, J. L. & Da Costa, E. (1934). *J. biol. Chem.* **107**, 351.
(10) Smeets, W. T. G. M. & Seekles, L. (1952). *Nature, Lond.*, **169**, 802.
(11) Pyne, G. T. (1953). *Proc. XIIIth Int. Dairy Congress, The Hague*, **3**, 1032.
(12) Van Krefeld, A. (1953). Discussion on foregoing. *XIIIth Int. Dairy Congress, The Hague.*
(13) Kass, J. P. & Palmer, L. S. (1940). *Industr. Engng Chem.* **32**, 1360.
(14) Gould, I. A. (1945). *J. Dairy Sci.* **28**, 367, 379.
(15) Pyne, G. T. & Ryan, J. J. (1950). *J. Dairy Res.* **17**, 200.
(16) Gould, I. A. & Frantz, R. S. (1945). *J. Dairy Sci.* **28**, 387.
(17) Howat, G. R. & Wright, N. C. (1934). *Biochem. J.* **28**, 1336.
(18) Eilers, H. (1945). Versl. *Rijkslandb Proefst., 's Grav.*, **50** (15), G 1009.
(19) Kometiani, P. A. (1931). *Milchw. Forsch* **12**, 433.
(20) Burg, P. van der (1947). *Ned. melk- en Zuiveltijdschr.* **1**, 69.
21) Pyne, G. T. (1949). *Proc. XIIth Int. Dairy Congress, Stockholm*, **2**, 231.

(*MS. received for publication* 6 *July* 1954)

637.127.681

573. THE TEMPERATURE COEFFICIENT OF EXPANSION OF RAW MILK

By A. L. SHORT

National Institute for Research in Dairying, University of Reading

(With 1 Figure)

An accurate method of determining the density of milk has been developed using Archimedes' principle of displacement. The method is capable of measuring a change of density to 1×10^{-5} g./ml., and it is intended to detect any changes in the density of milk which might be brought about by processing or other treatments.

The density of the processed and treated milks will be compared with that of raw milk. Complete data for the density of raw milk were not available, so the effect of composition and temperature of the density of raw milk was first investigated.

The investigation, therefore, has two aims: first to determine density as accurately as possible; and secondly, to establish relationships for temperatures between 10 and 45° C. between density and chemical composition, even though the latter cannot be determined with so high a degree of accuracy as the former.

Previous workers have dealt with various aspects of the expansion of milk. Whittaker, Sherman & Sharp (1) measured the density of skimmed milk over a temperature range 5–80° C. The composition of their skimmed milk was 8·96% solids, including 0·07% fat. Richmond (2) quotes values for the apparent expansion in glass of whole milk (3·8% fat) for a temperature range 30–80° F., and states that rich milk had a greater coefficient of expansion than poor milk. Hutchinson (3) examined the specific gravity of milks between temperatures 40 and 90° F. He calculated that compositional differences in milks of the same specific gravity at 60° F. would not affect the coefficient of expansion to any practical significance. Wegener (4) published results for whole and separated milk densities in temperature range 10–75° C., but did not give the effect of composition on the variation of density with temperature.

APPARATUS

The displacement method involves measuring the loss in weight of a sinker when placed in the fluid being examined. The sinkers were made of Pyrex glass in a torpedo shape and had a volume of approximately 50 ml. each. A fine suspension wire was sealed into one end of each sinker, and a chemical balance was converted so that a sinker could be suspended below the balance from one arm of the beam. The milk samples were examined in stainless steel cans which were placed in a constant-temperature bath controlled to 0·01° C. The temperature of the sample was checked by a differential thermocouple, one arm of the couple being placed permanently in the controlled bath and the other arm being placed in the sample can when it was put into the bath. A zero reading on a galvanometer connected to the thermocouple showed that there was no temperature difference between the sample and the control bath. The samples were stirred by the vertical movement of a stirrer which was made of two horizontal stainless steel vanes attached to a vertical shaft which was connected to a small motor by a nylon cord.

EXPERIMENTAL METHOD

Measurements of density were made at different temperatures at 5° C. intervals between 10 and 45° C. Above 45° C. the loss of water by evaporation from the surface of the sample was too great during the actual measurement to obtain repeatable results. The temperatures were applied in the following order: 20, 30, 40, 45, 35, 25, 15 and 10° C. The densities and compositions of the samples were measured at each constant temperature, and these samples were obtained 10 per day over 4 consecutive days.

The density was measured in duplicate for each sample using two sinkers, the density calculated from the simple formula

$$\text{density} = \frac{A - W_m}{V_t},$$

where A = absolute mass of sinker, W_m = weight in milk, V_t = volume of sinker at temperature to t° C.

The mass and volume of each sinker were calculated from weights of the sinkers in air and distilled water. The density of the air was calculated from humidity, pressure and temperature readings, the humidity being determined from wet and dry bulb thermometer readings. The density of distilled water was obtained from tables published in the *Handbook of Chemistry and Physics* (5).

Simple analyses of the samples were made and expressed in terms of fat and solid-not-fat percentages. The total solids were determined gravimetrically and the fat by the Gerber (7, 8) method, in replicate, using calibrated glassware. Gravimetric methods were not feasible for fat determination for such a large number of samples. Moreover, the relationships are entirely statistical and are derived from large numbers of readings.

All samples were initially warmed to 40° C. for 5 min. to ensure that the fat was in a melted condition during the density measurement as advised by the British Standards Institute (6). The temperature of the sample was then quickly adjusted to the working temperature required. Lids were fitted on the cans containing the samples until just before measurement to prevent loss of water by evaporation. Increase of density due to evaporation of water can be considerable: at 30° C. the rate of increase of density was observed to be 5×10^{-5} g./ml./hr. for a sample contained in a can from which the lid had been removed. The pH of samples was measured and samples of low pH rejected, the pH of samples accepted being between 6·4 and 6·7.

SAMPLING

In order to obtain a range of milk composition in which the fractions of fat and solids-not-fat themselves remain constant in composition, the following procedure was adopted. A 2-gallon sample of milk was obtained from evening-morning bulk of a herd of fifty cows. Half the sample was separated by machine and the cream added to the remaining whole milk, enriching it to about 6% fat. The separated and enriched milks were then mixed in different proportions to give ten samples in which the fat percentage ranged from 0·02 to 6·00% approximately, and the solids-not-fat from 9·3 to 8·4%. The artificial milks so made did not differ in density from natural milks of the same composition, as was shown in a subsidiary experiment. The densities of a natural milk and a separated milk were determined. They were then mixed accurately in simple proportions and densities of the mixtures measured. The density of each mixture was calculated from a knowledge of the

component densities and the volume of each in the mixture. This experiment was repeated for natural milk and enriched milk.

No significant difference between the calculated and the observed densities of the mixtures was found. This indicated that artificial milks composed as above can replace natural milks for density measurements. The results are shown in Table 1.

Table 1. *Density difference between natural milk and artificially composed milk*

Milk type	Density of milk observed			Calculated Density	Difference $\times 10^4$
	Sinker 1	Sinker 2	Mean		
Skim milk (U)	1·02869	1·02870	1·02870	—	—
Natural milk (V)	1·02545	1·02544	1·02545	—	—
$\frac{4}{5}$V + $\frac{1}{5}$U	1·02607	1·02608	1·02608	1·02608	0·0
$\frac{3}{5}$V + $\frac{2}{5}$U	1·02678	1·02680	1·02679	1·02674	0·5
$\frac{2}{5}$V + $\frac{3}{5}$U	1·02737	1·02738	1·02738	1·02739	−0·1
$\frac{1}{5}$V + $\frac{4}{5}$U	1·02803	1·02804	1·02804	1·02805	−0·1
Enriched milk E	1·01804	1·01804	1·01804	—	—
Natural milk F	1·02164	1·02160	1·02162	—	—
$\frac{1}{2}$E + $\frac{1}{2}$F	1·01875	1·01875	1·01875	1·01875	0·0
$\frac{3}{5}$E + $\frac{2}{5}$F	1·01950	1·01948	1·01949	1·01947	0·2

RESULTS

The density and solids-not-fat and fat percentages were obtained for each of forty samples at a constant temperature and a relationship between them calculated in the form

$$\text{density} = a\,(\text{S.N.F. }\%) + b\,(\text{fat }\%) + c.$$

a, b and c are constants for each temperature.

A relationship was obtained for each of the eight temperature levels between 10 and 45° C. The standard deviation for density calculated from each of the eight relationships varied between 1·5 and $2{\cdot}4 \times 10^{-4}$ g./ml. This was in good agreement with the accuracy expected. Both fat and solids-not-fat percentages were determined to 0·05% accuracy, and for solids-not-fat percentage, which is the more effective factor on density, the accuracy of 0·05% corresponds to an accuracy in density of 2×10^{-4} g./ml. at 20° C.

By substituting a value for fat and a value for solids-not-fat in each of the eight relationships a value for the density of milk of that particular composition can be calculated for each of the eight temperature levels. Values of fat percentages between 1 and 5% and values of solids-not-fat percentages between 8·6 and 9·1% were substituted in the relationship, thereby giving the density values of milks of such compositions between 10 and 45° C.

Skimmed milk was considered separately throughout the calculations.

The density was plotted against the temperature for all the milk compositions studied and cubic equations calculated to give the best fit to the experimental curves by the method of least squares, i.e.

$$\text{density} = a + bt + ct^2 + dt^3.$$

a, b, c and d are constants for each composition.

A typical composition of a natural milk used in the experiments was 8·70% solids-not-fat and 3·00% fat. A skimmed milk composition could be represented by 8·90% solids-not-fat and 0·02% fat. The density-temperature relationships of these milks were as follows:

$$\text{Full milk density} = 1{\cdot}0350 - 0{\cdot}000358t + 0{\cdot}0000049t^2 - 0{\cdot}00000010t^3,$$

$$\text{Skimmed milk density} = 1{\cdot}0366 - 0{\cdot}000146t + 0{\cdot}0000023t^2 - 0{\cdot}00000016t^3,$$

where t = temperature in ° C.

Variation of expansion with composition

The coefficient of expansion of milk increases with both fat and solids-not-fat percentage composition. Table 2 shows the variation in the constants of the cubic equation. In Table 2*a* the variation for variable fat content is given for three levels of solids-not-fat composition. The increase of the coefficients is regular and curves relating density and temperature are shown in Fig. 1*b* for milk compositions 1, 3 and 5% fat, each having 8·8% solids-not-fat.

Table 2*a*. *Comparison of equation constants for varying fat percentage composition.* S.N.F. *percentage constant*

$D = a - bt + ct^2 - dt^3$; $D = (\text{density} - 1) \times 10^4$; $t = $ temp. ° C.

Fat (%)	*a* S.N.F. (%)			*b* S.N.F. (%)			*c* S.N.F. (%)			*d* S.N.F. (%)		
	8·6	8·8	9·0	8·6	8·8	9·0	8·6	8·8	9·0	8·6	8·8	9·0
1	357·4	365·0	372·3	2·65	2·82	2·97	0·0374	0·0352	0·0322	0·00100	0·00091	0·00083
2	352·4	367·4	367·4	3·01	3·21	3·33	0·0424	0·0417	0·0372	0·00101	0·00094	0·00084
3	348·0	355·4	362·8	3·44	3·59	3·75	0·0504	0·0473	0·0450	0·00105	0·00096	0·00088
4	342·9	350·4	358·1	3·79	3·95	4·13	0·0548	0·0523	0·0507	0·00106	0·00097	0·00090
5	338·1	345·8	353·3	4·18	4·37	4·52	0·0615	0·0601	0·0570	0·00109	0·00101	0·00092
Average difference per 1% fat		4·8			0·39			0·0061			0·00002	

Table 2*b*. *Comparison of equation constants for varying* S.N.F. *percentage. Fat percentage constant*

$D = a - bt + ct^2 - dt^3$; $D = (\text{density} - 1) \times 10^4$; $t = $ temp. ° C.

Fat (%) / S.N.F. (%)	*a*			*b*			*c*			*d*		
	2	3	4	2	3	4	2	3	4	2	3	4
8·6	352·4	347·9	342·9	3·01	3·44	3·78	0·0424	0·0504	0·0548	0·00101	0·00105	0·00106
8·7	356·5	351·6	347·1	3·14	3·51	3·92	0·0435	0·0485	0·0559	0·00100	0·00100	0·00104
8·8	360·2	355·4	350·4	3·21	3·59	3·95	0·0420	0·0470	0·0523	0·00095	0·00095	0·00097
8·9	363·7	358·8	354·3	3·27	3·63	4·05	0·0395	0·0446	0·0519	0·00089	0·00090	0·00094
9·0	367·4	362·8	358·1	3·33	3·75	4·13	0·0370	0·0449	0·0510	0·00084	0·00088	0·00090
9·1	371·5	366·5	362·1	3·47	3·81	4·25	0·0387	0·0428	0·0507	0·00083	0·00082	0·00087
Average difference per 0·1% S.N.F.		3·8			0·08			Very variable, but decreasing			−0·00004	

The variation of coefficients for varying solids-not-fat content at constant fat percentage is given in Table 2*b*. The experimental results show a regular increase in the linear term only. The overall effect of the solids-not-fat content is more definitely illustrated in Fig. 1*a*.

It can be seen from Fig. 1 that the density-temperature relationship approximates closely to linearity between temperatures 10–30° C. for fat percentages over 3%. The linearity extends above 30° C. with increasing fat percentage. Using the form

$$D = n - mt,$$

where $D = (\text{density} - 1) \times 10^{-4}$, $t = $ temperature in ° C., values for m have been calculated for varying compositions and these are given in Table 3.

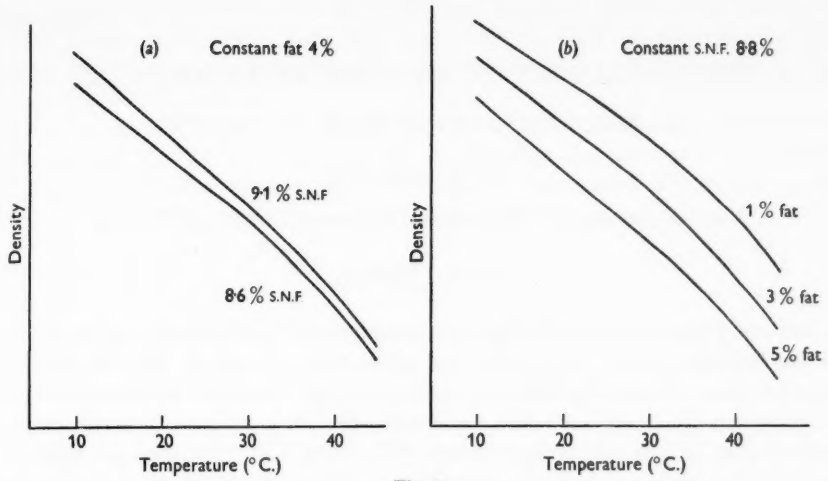

Fig. 1

Table 3. *Temperature coefficient of density for varying compositions in temperature range* 10–30° C.

$D = n - mt$; D = density; t = temp. ° C.

	Values of m Fat (%)		
S.N.F. (%)	3	4	5
8·6	0·000280	0·000295	0·000310
8·7	290	300	
8·8	295	310	330
8·9	300	320	
9·0	305	330	345
9·1	315	335	

SUMMARY

The density of raw milk between 10 and 45° C. has been examined. The effect of composition on the expansion of raw milk has been studied and the variation of the density-temperature coefficient quoted for different values of milk composition.

I am indebted to Dr S. J. Rowland for his advice and to Mr J. H. Prentice and Mr L. W. Phipps for helpful discussions. I wish to thank Miss H. R. Chapman and her staff for preparing the samples.

REFERENCES

(1) WHITTAKER, R., SHERMAN, J. M. & SHARP, P. F. (1927). *J. Dairy Sci.* **10**, 361.
(2) RICHMOND, H. D. (1953). *Dairy Chemistry*, p. 110. London: C. Griffin and Co. Ltd.
(3) HUTCHINSON, R. C. (1940). *J. Aust. Inst. agric. Sci.* **6**, 205.
(4) WEGENER, H. (1953). *Milchwissenschaft*, **8**, 433.
(5) *Handbook of Chemistry and Physics* (1953). P. 1114. Cleveland, Ohio, U.S.A.: Chemical Rubber Publishing Co.
(6) British Standards Specification (1937). No. 734.
(7) British Standards Institution (1936). B.S. 696, pt. 2, p. 9.
(8) British Standards Institution (1951). B.S. 1741, p. 7.

(*MS. received for publication* 20 *July* 1954)

637.128.2

574. COLOUR CHANGES IN HEATED AND UNHEATED MILK

II. THE WHITENING OF MILK ON HEATING

By H. BURTON

National Institute for Research in Dairying, University of Reading

(With 7 Figures)

In a previous paper (1) the use of reflectance measurements to investigate the browning of milk was discussed, and it was shown that on heating a sample of milk the reflectance rises at first and subsequently falls as browning develops. The initial rise, in common with the subsequent fall, is obtained with whole milk whether homogenized or not, and with separated milk, but to only a slight extent with cream. The purpose of this paper is to show that the rise in reflectance is caused by a reaction independent of the browning reaction responsible for the fall in reflectance, to report some characteristics of the increase in reflectance, and to discuss the reasons for its occurrence. The methods used for the heat treatment of samples and for the determination of reflectance were those described in the previous paper unless otherwise stated.

THE INDEPENDENCE OF THE BROWNING AND WHITENING REACTIONS

A typical curve showing the reflectance of a sample of separated milk when heated for various times is shown in Fig. 1, curve *a*. This curve is taken from Fig. 7 of the previous paper of this series (1), and gives the variation in reflectance at a wave-length of 4260 Å. of separated milk held at 100° C. On chemical grounds one might have expected the browning to proceed normally with time to give an exponential fall in reflectance (curve *b*). For the

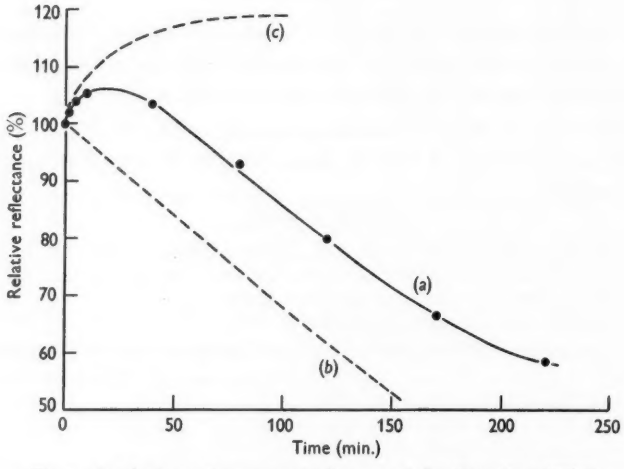

Fig. 1. A reflectance-time curve (*a*) for separated milk held at 100° C., and the components of the curve (*b*) and (*c*).

shape of the experimental curve *a* to be obtained a second component must have been present, giving a rise in reflectance with time (curve *c*). From the shape of curve *a*, the whitening (as the rise in reflectance can be called) must have occurred rapidly, but must soon have reached a maximum.

It was not difficult to obtain experimental evidence for the independent existence of the whitening and the browning reactions. The trypsin-digestion method of Choi, Koncus, O'Malley & Fairbanks (2) might be expected to give a true measure of the development of

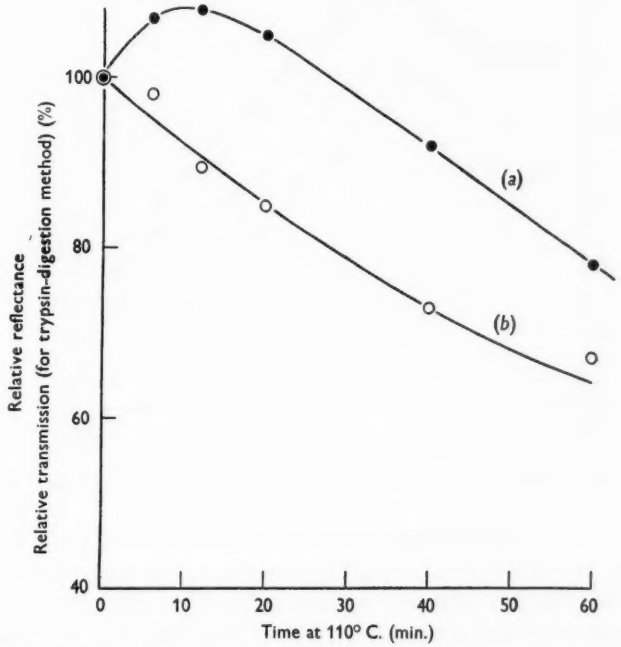

Fig. 2. A comparison of the effect of heat on (*a*) the reflectance of separated milk, and (*b*) the brownness of the same milk as determined by the trypsin-digestion method.

the brown pigment in the milk. Concurrent measurement of the reflectance of samples of separated milk heated for different times at 110° C., and of the brownness of the same samples determined by the trypsin-digestion method gave the results shown in Fig. 2. The trypsin-digestion method therefore gives a result which is uninfluenced by the rise in reflectance and which is similar to curve *b* of Fig. 1.

It was also found that whitening could be obtained without browning. The addition of formaldehyde to suppress the browning (1) did not permit a satisfactory demonstration of this, since the whitening mechanism was also affected. However, whitening was obtained at temperatures below 100° C., at which the browning occurs only extremely slowly or not at all. Reflectance curves similar to curve *c* of Fig. 1 were then obtained. The whitening also differs from the browning in its effect on the spectral variation of reflectance. The fall in reflectance which constitutes browning is slight at the red end of the visible spectrum, but becomes progressively greater towards the blue and ultra-violet parts of the spectrum (1). On the other hand, the rise in reflectance which constitutes whitening was found

to be constant throughout the visible range and up to at least 12,000 Å. wave-length in the near infra-red. Fig. 3 shows the reflectance relative to opal glass throughout the visible spectrum of samples of separated milk which were heated for various times at 80° C. It is clear, therefore, that whitening and browning are independent phenomena brought about by different reactions.

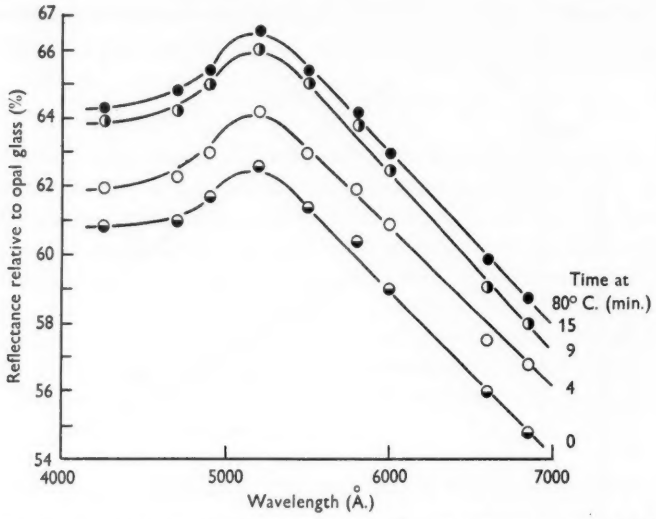

Fig. 3. The spectral distribution of reflectance for milk with different degrees of whitening.

CHARACTERISTICS OF WHITENING REACTION

Temperature. It was found to be difficult to obtain reproducible results for the rise in reflectance when milk was subjected to specific time-temperature treatments. The differences between individual samples of milk were such that they were clearly brought about by differences in the milk composition. The same sample of milk did not give reproducible results unless precautions were taken, and where small reflectance changes are of significance, non-reproducibility within even narrow limits can invalidate the results. Measurement of reflectance at constant temperature and control of the time-temperature history of the sample between heat treatment and measurement did much to eliminate the variations but some slight unexplained variations still remained.

Fig. 4 shows the whitening at different temperatures of two typical samples of separated milk. The reflectance measurements were made with an EEL spectrophotometer at a wave-length of measurement of 4260 Å., relative to a standard of Phillips's 'Milk of Magnesia'* contained in an optical cell. The whitening is expressed as the absolute rise in reflectance above that for unheated milk, since as discussed earlier this rise is the same for all wave-lengths. As shown by Fig. 4, whitening is a function of time and temperature, the temperatures involved being much lower than those causing browning. While whitening does not occur at 60° C. it becomes quite measurable at 70° C. The curves relating whitening with time are not exponential as would be obtained with a simple

* Phillips's 'Milk of Magnesia' is a convenient short-term standard, with a reflectance closer to that of milk than is the reflectance of opal glass.

first-order reaction. Fig. 4*a* appears to indicate that the maximum amount of whitening depends upon the temperature at which the milk is held; the higher the temperature the higher the reflectance to which the curve is asymptotic. However, Fig. 4*b* has a more extended time scale, and suggests that if the milk is held for a long period the reflectance may continue to rise slowly to approach a common maximum. The difference in the

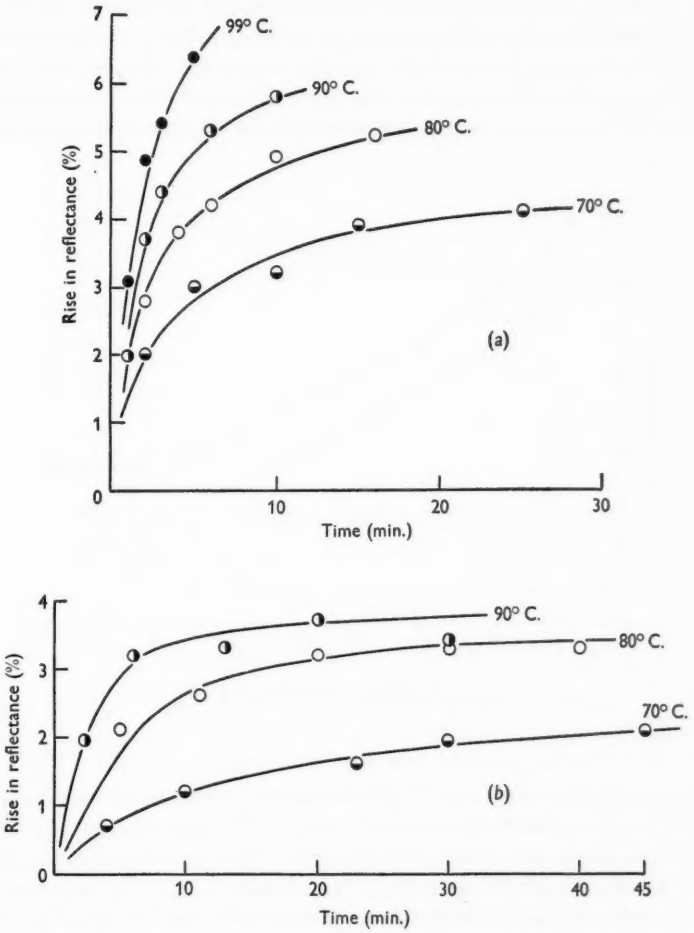

Fig. 4. The effect of time and temperature of holding on the reflectance of two samples of separated milk. (*a*) sample 1, (*b*) sample 2.

magnitude of the reflectance changes in these two cases is typical of those which were obtained with different samples of milk. Because of the complex nature of the curves, it is difficult to draw any conclusions as to the thermal characteristics of the reaction, but from the early part of each curve a Q_{10} of between 2 and 3 seems probable.

pH. The pH values of different samples from the same bulk of milk were adjusted by the addition of sulphuric acid or sodium hydroxide. The samples were then heated for

4 min. and for 15 min. at 90° C. The reflectances relative to opal glass were measured at a wave-length of 4260 Å., and the rises in reflectance above those of unheated samples of the corresponding pH were determined. The results are given in Fig. 5. The two treatment durations were chosen to correspond to points on the early part of the whitening curve, and to the maximum amount of whitening that would be reached. The two curves in Fig. 5 therefore indicate the effect of pH on both the rate of whitening and the maximum whiteness. It can be seen that both are influenced by pH in the same way, a lower pH giving more rapid whitening and a higher final reflectance. These results agree with those given in Fig. 9 of the first paper of this series (1) in which the effect of pH on browning is shown. The early parts of the curves in that figure show that pH not only affects the rate of browning but also influences the maximum reflectance reached, in the sense obtained above.

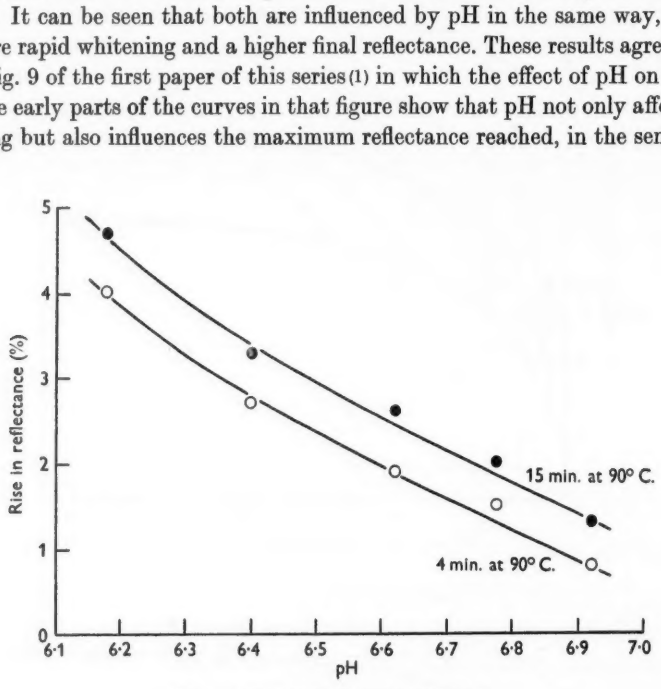

Fig. 5. Variation of whitening with pH.

THE CAUSE OF WHITENING

The fact that whitening consists of a uniform increase in reflectance over a wide range of wave-lengths suggests that the phenomenon may be brought about by an increase in the numbers of optically opaque particles in the milk. This, together with the temperature range over which the whitening occurs, immediately suggests a connexion with the denaturation and coagulation of the soluble proteins, albumin and globulin. Rowland (3) has shown that denaturation of the albumin and globulin of milk begins at about 60° C. and continues with increasing rapidity at higher temperatures. The curves which he gives relating degree of denaturation with time for temperatures of 65–75° C. show a strong resemblance in shape to the curves of Fig. 4. In a subsequent paper by the same author (4), the data on denaturation were extended to higher temperatures. A plot on logarithmic co-ordinates of the figures given in that paper shows that the denaturation is far from exponential, particularly at temperatures below 90° C. The slopes of the logarithmic plots over the early parts of the denaturation curves indicate a Q_{10} of 2–3 for temperatures between 75 and 90° C., and this figure agrees well with that found over a similarly restricted

range of conditions for whitening. However, as pointed out by Rowland, the heat coagulation of the soluble protein in milk consists of two stages: heat denaturation is followed by the precipitation of the denatured protein under suitable conditions. Only the final precipitation of denatured protein in particles large enough to be optically opaque can be expected to influence the reflectance of the milk. Of the factors which might influence the degree of coagulation, pH is the most important. A curve showing the effect of pH on the amount of denatured protein coagulated after 10 min. at 100° C. has been given by Rowland (5), and the part of that curve lying between pH 6 and 7 is similar to the curves in Fig. 5. In both cases the curves are almost linear and show an increase with decreasing pH. The reflectance of the heated milk therefore varies with pH in the same way as the amount of denatured, coagulated albumin and globulin might be expected to vary.

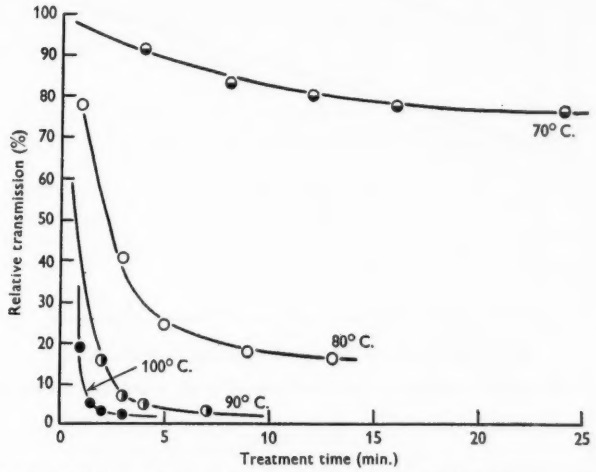

Fig. 6. Variation of the opacity of rennet whey with time and temperature.

Two groups of experiments were performed to investigate directly the association between whitening and heat coagulation of the soluble proteins of milk. In the first, rennet whey (pH 6·50) was prepared from a sample of separated milk. The whey was diluted 1:1 with distilled water and was heated for various times at temperatures corresponding to those used in the whitening determinations. The relative light transmissions of the heated whey were determined by means of a Hilger 'Biochem' absorptiometer using a 1 cm. optical cell. The results are shown in Fig. 6. There is a strong resemblance between the curves of Fig. 6 and those of Fig. 4, the proportion of transmitted light being an inverse function of reflectance. The increase in the content of optically opaque material in milk due to heat coagulation of the soluble protein thus shows the same characteristics as the increase of whiteness on heating.

In the second group of experiments the soluble-protein content of milk was artificially raised by the addition of soluble protein, and the whitening on heating was then determined. Whey was prepared from separated milk by the addition of acetic acid followed by sodium acetate (6), and filtration. The whey was dialysed against running tap water for 24 hr. and against distilled water for 24 hr.; the pH was then 6·2. The dialysed whey was freeze-dried. Different weights of the freeze-dried material were added to 10 ml. portions

of fresh separated milk in which they dissolved easily, and the reflectances of these portions were measured relative to opal glass before and after heating at 90° C. for 15 min. Addition of the freeze-dried soluble protein caused a drop in the reflectance of the unheated milk and an increase in the reflectance of the heated milk proportional to the quantity added. For two different milks, the effect of the addition of different weights on the rise of reflectance is given in Fig. 7. These results show that the amount of whitening of milk on heating depends directly on the soluble-protein content of the milk.

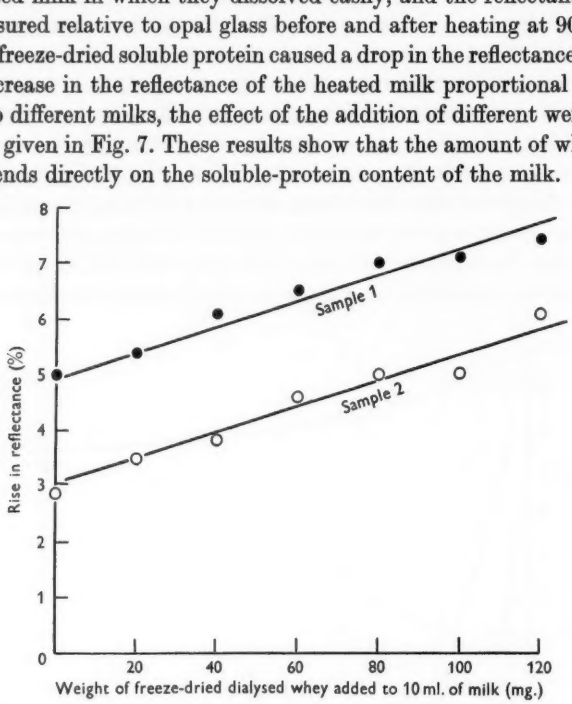

Fig. 7. The effect of additions of soluble proteins on the whitening of separated milk.

CONCLUSIONS

The colour of milk undergoes two independent changes during heating. In addition to the browning at high temperature discussed previously, there is a whitening which occurs at temperatures above 60° C. and which consists of a general rise in reflectance throughout the visible spectrum. The whitening is caused by denaturation and subsequent coagulation of the soluble-protein component of the milk, which increases the content of optically opaque particles in the milk.

The amount of whitening varies directly with the soluble protein content, and also increases with decreasing pH. Different samples of milk can therefore show widely differing amounts of whitening.

It is possible that the maximum amount of whitening increases with the temperature at which the milk is held.

Because of the complex character of the whitening curves, which are not exponential, accurate figures cannot be given for the variation of whitening with temperature, but a Q_{10} of between 2 and 3 seems to be indicated.

SUMMARY

In previous work the browning reaction in milk was examined by measuring the fall in optical reflectance which occurred during browning. It was then found that the heating of milk caused an initial rise in its reflectance, and, in the present paper this rise in reflec-

tance, or whitening of milk, is examined in more detail. It is shown that the reaction causing it is independent of the subsequent browning. Whitening occurs at temperatures above 60° C., and takes place more rapidly at higher temperatures. The curves of reflectance against time are not exponential, and there is some evidence that the maximum amount of whitening depends on the temperature at which the milk is held. Reduction in pH of the milk causes an increase in the amount of whitening for a particular time-temperature treatment. From these data and other experiments it is concluded that the rise in reflectance is brought about by the denaturation of the soluble proteins of the milk and their subsequent coagulation into particles large enought to reflect light.

Much of the experimental work described in this paper was carried out by Mr Tom Cheeseman, and I would like to express my thanks to him for his assistance. I am also indebted to Dr S. J. Rowland and Dr R. Aschaffenburg for the valuable discussions I have had with them.

REFERENCES

(1) BURTON, H. (1954). *J. Dairy Res.* **21**, 194.
(2) CHOI, R. P., KONCUS, A. F., O.MALLEY, C. M. & FAIRBANKS, B. W. (1949). *J. Dairy Sci.* **32**, 580.
(3) ROWLAND, S. J. (1933). *J. Dairy Res.* **5**, 46.
(4) ROWLAND, S. J. (1937*a*). *J. Dairy Res.* **8**, 1.
(5) ROWLAND, S. J. (1937*b*). *J. Dairy Res.* **8**, 6.
(6) ROWLAND, S. J. (1938). *J. Dairy Res.* **9**, 42.

(*MS. received for publication* 21 *July* 1954)

637.128.2

575. COLOUR CHANGES IN HEATED AND UNHEATED MILK

III. THE EFFECT OF VARIATION IN MILK COMPOSITION ON THE WHITENING AND BROWNING OF SEPARATED MILK ON HEATING

By H. BURTON and S. J. ROWLAND

National Institute for Research in Dairying, University of Reading

(With 7 Figures)

INTRODUCTION

Two previous papers in this series (1, 2) have described the rise and subsequent fall in the reflectance of milk which occurs on heating. The rise in reflectance is caused by the denaturation and coagulation of the soluble proteins of the milk, and the fall in reflectance is the result of the formation of a brown pigment in the milk from lactose-protein interaction in the 'browning' or Maillard reaction. Some indication of the factors influencing the magnitude of the colour changes due to these two reactions has also been given. However, the experiments described previously were concerned with artificially induced changes in pH and milk composition. They did not show how normal variations in these factors would affect the shape of the reflectance-time curve, the maximum whitening and rate of browning, and the final colour after a certain time-temperature treatment. The variation with milk composition of this last characteristic, may be of great commercial importance in the production of sterilized and evaporated milks.

Extreme examples of variations in milk composition are obtained by using the milk from individual cows of different breeds and at different stages of lactation. Variations in bulk milk composition are much less than those shown by individual cows. At the same time, seasonal variations in bulk milk composition can be accounted for to some extent by general changes in the stage of lactation of large numbers of animals. Experiments on milk from individual cows have been used therefore to give information on the variations in the susceptibility to whitening and browning of bulk milk supplies.

EXPERIMENTAL

Source and treatment of milk samples

The experimental milk samples were taken from individual cows in the Institute herds. Three breeds were available, Guernsey, Friesian and Shorthorn, and the animals selected from each breed were chosen to represent all stages of lactation from early to late, excluding only the colostral stage.

Three groups of experiments were carried out. In the first, milk was taken from four Guernsey, four Friesian and four Shorthorn cows; the second comprised milk from four Friesians and four Shorthorns; and the third milk from three Guernseys, three Friesians and three Shorthorns. The general experimental procedure was as follows. A sample of approximately 1 pint was taken from the milk of each cow and was separated in a small mechanical separator. 7 ml. samples of each cow's milk were heated in metal screw-top tubes in an oil-bath at 110·0° C. for 20, 40, 60, 80 and 100 min. for the first group of sam-

ples and for 10, 20, 40, 60 and 80 min. for the other groups. The samples were then cooled immediately to room temperature and held at 20° C. before measurement of reflectance. The reflectances of the heated samples of milk and of corresponding unheated samples were determined relative to opal glass at a wave-length of 4260 Å. by means of an EEL reflectance spectrophotometer as described in the first paper of this series (1).

Analyses

The pH and titratable acidity of the unheated separated milk from each cow were determined at the time of heat treatment. For the first and second groups of samples the unheated milk from each cow was tested for total solids gravimetrically, fat by the Gerber method and hence solids-not-fat by difference, lactose by a modification of the chloramine-T method of Hinton & Macara (3), and total nitrogen, casein nitrogen and soluble protein nitrogen by the method of Rowland (4). In the third group of samples, only restricted analyses sufficient to determine the nitrogen distribution were made.

RESULTS AND DISCUSSION

The absence of any samples with a 10 min. heating time in the first group made the determination of whitening for this group unreliable, and with restricted analyses available for the third group of samples, a complete series of results was available only for the second group. These results are therefore the only ones presented in detail, and they are given in Fig. 1 and Table 1. However, the subsequent discussion makes use of all the available data, i.e. rate-of-browning figures from all three groups (29 samples), whitening figures from the second and third groups (17 samples), full chemical analyses from the first and second groups, and partial analyses from the third group.

Table 1. *The composition of the second group of milk samples with results for the maximum rise of reflectance and maximum rate of browning*

Name of cow ...	Flora 107	Rosalie 38	Campion 29	Peace 34	Regina	Venture	Bramble	Bugle
Breed ...	Shorthorn	Shorthorn	Shorthorn	Shorthorn	Friesian	Friesian	Friesian	Friesian
Approx. time since calving months ...	11	10	3	1	11	10	2	2
Milk analysis (%)								
Fat	0·15	0·08	0·05	0·02	0·10	0·05	0·05	0·05
Solids-not-fat	9·85	9·01	8·83	9·18	9·70	9·51	9·92	9·15
Lactose	4·74	4·74	4·87	4·96	4·88	4·53	4·88	5·06
Protein (total nitrogen × 6·38)	4·12	3·42	2·99	3·25	3·92	4·04	3·33	3·20
Ash	0·78	0·76	0·70	0·77	0·75	0·75	0·76	0·71
Nitrogen distribution (mg./100 g.)								
Total nitrogen	646	536	469	509	614	633	523	502
Casein nitrogen	513	426	370	390	486	516	403	389
Soluble-protein nitrogen	101	76	71	90	97	88	91	85
Non-protein nitrogen	32	34	28	29	31	29	29	28
Casein number*	79·4	79·4	78·9	76·6	79·1	81·5	77·0	77·5
pH	6·45	6·66	6·57	6·45	6·64	6·55	6·47	6·60
Titratable acidity	0·150	0·130	0·150	0·180	0·130	0·145	0·175	0·130
Maximum browning rate (% in 5 min.)	3·4	3·6	3·9	4·2	3·3	3·25	3·75	4·05
Maximum reflectance rise (%)	9·0	5·5	5·4	6·0	5·0	6·5	6·3	4·0

* Casein number = casein nitrogen × 100/total nitrogen (6).

Fig. 1 shows the variation of reflectance with time for the eight samples of the second group. The reflectances are expressed as a percentage of the reflectance of the corresponding unheated sample. The relevant chemical data are given in Table 1. The reflectance curves have the normal form of a rise followed by a fall which is the first part of an exponential drop in reflectance to a low value. The rate of browning has been taken as the slope of the curve after the initial rise of reflectance is complete. With some curves the

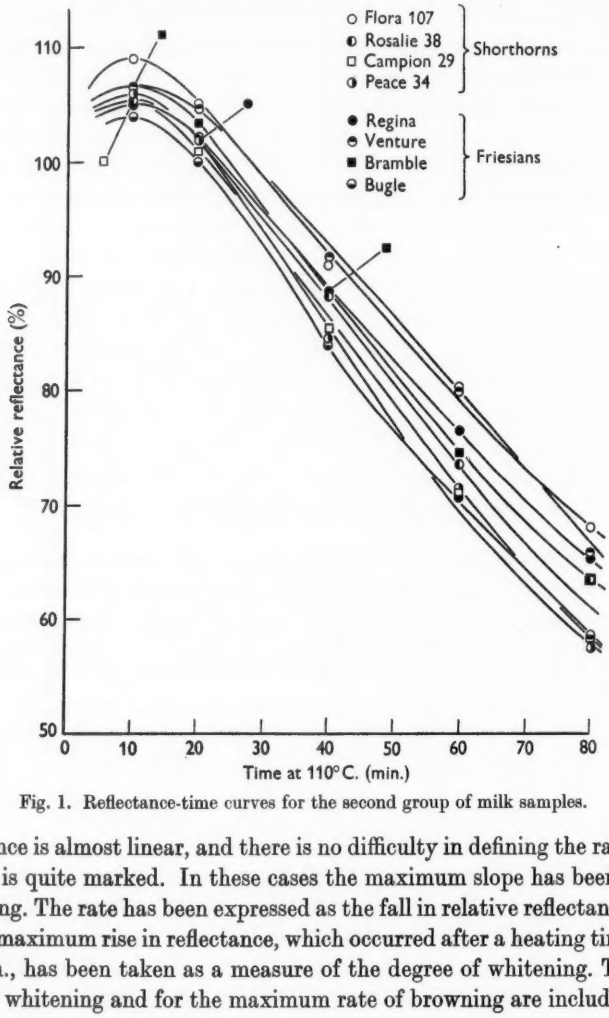

Fig. 1. Reflectance-time curves for the second group of milk samples.

fall in reflectance is almost linear, and there is no difficulty in defining the rate. In others, the curvature is quite marked. In these cases the maximum slope has been taken as the rate of browning. The rate has been expressed as the fall in relative reflectance for a 5 min. interval. The maximum rise in reflectance, which occurred after a heating time of approximately 10 min., has been taken as a measure of the degree of whitening. The figures for the maximum whitening and for the maximum rate of browning are included in Table 1.

The effect of variation in milk composition on the rate of browning

The factors which might be expected to influence the rate of browning of a sample of milk are (i) the pH of the sample, since this has been found previously to have a considerable effect, and (ii) the content of those constituents which might be involved in the browning reaction, e.g. proteins and lactose.

The relation between the maximum rate of browning and the pH of the untreated milk is shown for all the samples in Fig. 2. Included in this figure is the curve obtained previously(1) for the variation in the rate of browning of samples of the same bulk of milk in which the pH was artificially adjusted. The agreement between the distribution of the points for the natural milk samples and the shape of the curve is good, although the curve lies to the lower side of the distribution.

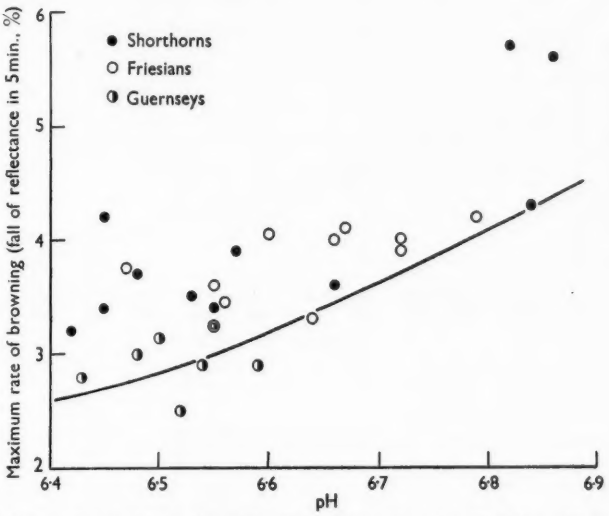

Fig. 2. Variation of rate of browning with pH for all sample. —— Variation of browning rate for a sample of milk artificially adjusted to different pH's (1).

The variations of the rate of browning with casein, soluble protein and lactose contents are shown in Fig. 3*a–c*. There appears to be no connexion between the rate of browning of a sample and the amount of soluble protein and lactose present in the sample. In the distribution for lactose (Fig. 3*c*), the three points corresponding to a low lactose content are rather higher than might be expected, but these particular milks were also samples of high pH, and showed a high rate of browning for that reason. The rate of browning declined steadily with increasing casein content (Fig. 3*a*), a result which at first seems contrary to what might be expected, but which can readily be explained.

The assessment of the effect on the rate of browning of variations in the amount of the individual constituents in the milk is complicated by the tendency for the natural variations in these constituents to be highly correlated. Thus, with advancing stage of lactation, there is normally an increase in the casein content of the milk, a relatively greater increase in the soluble proteins, a decrease in lactose, and an increase in the pH value. There is often a similar general interrelation of these factors in the milk from different cows.

If the casein content of the experimental milk samples is expressed in terms of the 'casein number' (casein nitrogen × 100/total nitrogen(5)), a linear relation is obtained between rate of browning and casein number as shown in Fig. 4. A corresponding inverse relationship must also exist with the proportion of soluble protein. A decreasing casein number is usually accompanied by an increasing pH; the association between these two factors for the range of milks used in these experiments is clearly shown in Fig. 5. It

therefore seems probable that the variation of the rate of browning with casein content and with casein number is only a further aspect of the effect of pH on the rate of browning. Moreover, as shown in Fig. 2, the browning of different samples of natural milk varied with pH in the same way as the browning of a milk of fixed composition but of artificially adjusted pH. The natural milks therefore behaved as if their chemical compositions, but not their pH values, were the same. We know, however, that the compositions were not

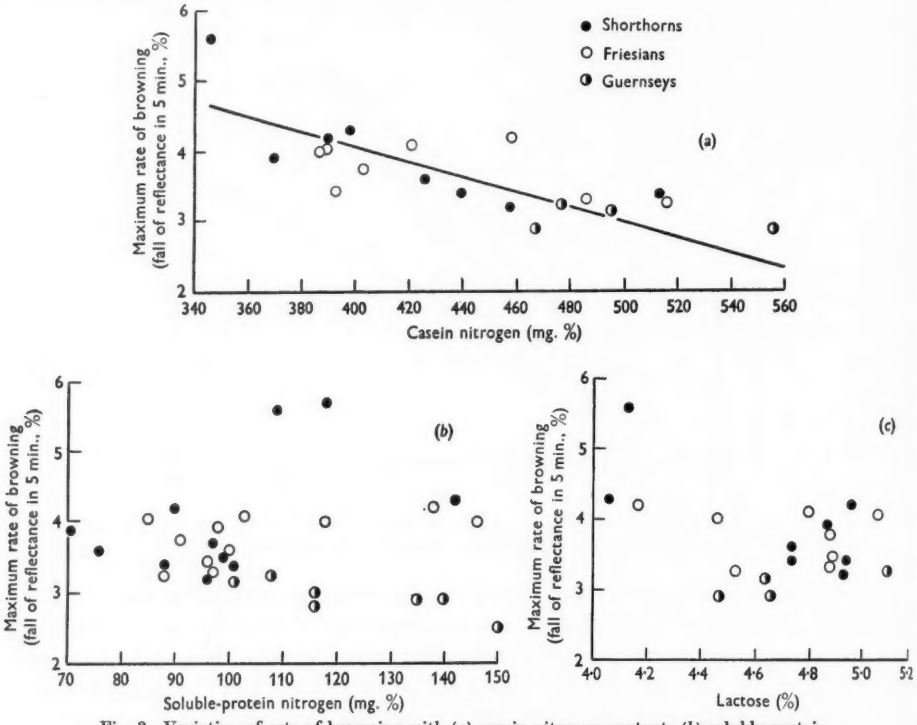

Fig. 3. Variation of rate of browning with (*a*) casein nitrogen content; (*b*) soluble-protein nitrogen content; (*c*) lactose content.

the same, so that the variations in composition appear to affect the rate of browning only in so far as they influence the pH. That the rate of browning is not increased by increase in the absolute amounts of casein, soluble protein and lactose present in the milk is consistent with the amounts of these compounds entering into the browning reaction being but a small proportion of the total amounts present. The rate at which the reaction proceeds at constant temperature is then controlled by the pH or by some other characteristic which itself influences the pH.

The effect of variation in milk composition on whitening

Earlier results(2) have shown that the whitening of samples of natural milk might be expected to be influenced by the pH of the samples. Also in view of the previous identification of the soluble proteins of milk as the source of whitening, the amount of whitening might be expected to be influenced by the soluble protein content. The variation of the

maximum whitening with pH for all the samples for which the data are available, comprising the second and third groups of samples, is shown in Fig. 6. Also shown in this figure is the curve previously obtained (2) for the variation of the maximum reflectance reached when samples of a common bulk of milk were artificially adjusted in pH. Although

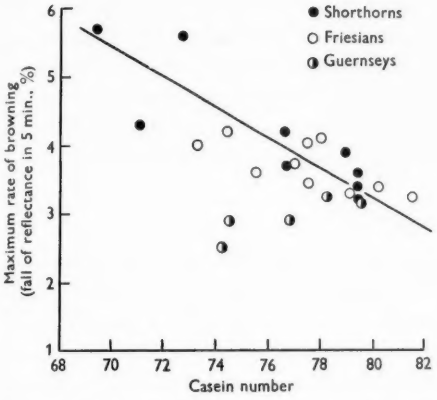

Fig. 4. Variation of rate of browning with casein number.

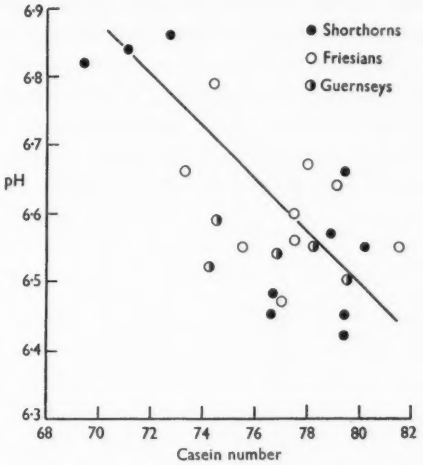

Fig. 5. Variation of pH with casein number.

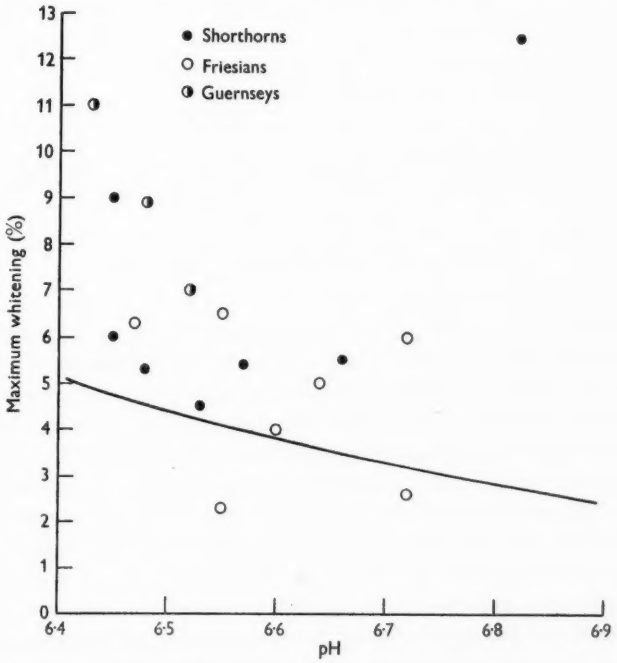

Fig. 6. Variation of maximum whitening with pH for the second and third groups of samples.
—— Variation of maximum whitening for a sample of milk artificially adjusted to different pH's (2).

there was a general trend towards increasing whitening with decreasing pH, the agreement with the previous curve is poor. Isolated points differ considerably from the mean, particularly in the case of the sample with pH 6·82.

The variation of whitening with the soluble-protein nitrogen content is shown in Fig. 7. The scatter of the points here is even more pronounced than with the pH results in Fig. 6. The calculated regression line, which is shown on the figure, has a slope of 0·0487 with a standard error of 0·0335: this slope is significant only at about the 17% level.

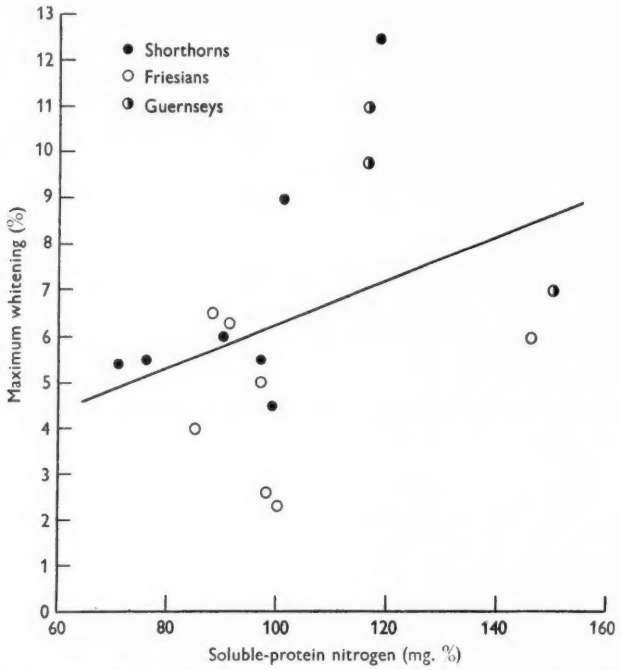

Fig. 7. Variation of maximum whitening with soluble-protein content for the second and third groups of samples. —— Calculated regression line.

The presentation of the whitening results on the basis of increase in relative reflectance, although not the best method, has been adopted here to avoid expressing the results in different ways when discussing whitening and browning. Since the whitening consists of a uniform increase in reflectance throughout the visible spectrum, the absolute increase in reflectance might have been expected to give better agreement with the pH and soluble-protein results than did the relative increase. In fact, owing to the wide experimental variation, the agreement was no better when the results were presented as a function of the absolute increase, and the slope of the regression line relating whitening with soluble protein had the same degree of significance as before.

Previous results in which the amount of soluble protein in milk was artificially increased (2), when converted to refer to relative reflectances and soluble protein content expressed as mg.N/100 g., indicated a good linear relationship between whitening and soluble-protein content with a slope of approximately 0·03. This is well within the limits

of error of the slope in Fig. 7. The low level of significance obtained with different samples of milk must therefore indicate that there is some other characteristic of the milk composition which has a marked effect on the degree of whitening and which is not apparent from the chemical analyses.

It was shown above that the apparent variation of rate of browning with chemical composition was in fact a reflexion of the effect of pH on browning. There is no question, however, of the variation of whitening with soluble-protein content being similarly linked with the effect of pH. Increase in soluble-protein content would in general be accompanied by an increase in the pH of the milk because of its effect on the protein ratios, this increase in pH resulting in a decrease in the amount of whitening. This is the reverse of the effect which is actually found, so that the effects of pH and of soluble-protein content are independent. This is in agreement with the concept of whitening being caused by denaturation and subsequent coagulation of the soluble proteins.

CONCLUSIONS

The results for both browning and whitening support those obtained previously on samples of milk with artificially adjusted pH and soluble-protein content. The rate of browning is not affected by the proportion of milk constituents, except in so far as these are associated with changes in the natural pH of the milk. Over the whole range of samples the variation in the rates of browning is only about 2:1. The whitening of the different samples shows the expected general relationship with pH and soluble-protein content. The variations in the results are so wide, however, that they indicate the presence of some important factor or factors not covered by the chemical examination that was made.

There is a considerable range of reflectances, and hence of colours, of the milk samples after any heat treatment. These differences are determined much more by variations in the amount of whitening, which cover a range of about 5·5:1, than by variations in the rate of browning. It follows that the colour of heated milk is influenced to a great extent by the individual characteristics of the milk itself. In practice the variations in bulk milk will not be as great as in the samples of milk used for these experiments which came from individual cows.

SUMMARY

The effect of natural variations in the pH, and in the casein, soluble-protein and lactose contents on the whitening and browning of separated milk has been studied by holding samples of known composition, obtained from individual cows, at a temperature of 110° C. for various times and determining their reflectances after treatment.

The rate of browning, measured as a rate of loss of reflectance at a wave-length of 4260 Å., was found to depend on the pH of the milk. Apparent variations of the rate of browning with protein content are considered to have been caused by associated variations of pH with protein content in the milk from the different cows. The rate of browning varied over a range of 2:1 for the samples examined.

The whitening of milk, measured as the maximum rise of reflectance occurring on heating, was found to be much more variable, and over the same range of samples varied by 5·5:1. The soluble-protein content and pH affect the amount of whitening, but there appears to be some other factor of importance which was not covered by the chemical analyses that were made.

A considerable range of reflectances is shown by different milk samples after a fixed heat treatment. These differences are determined more by variations in the amount of whitening than by variations in the rate of browning.

We would like to express our thanks to Mrs R. E. Winder and Mr A. W. Wagstaff for their help with the analyses and to Mr Tom Cheeseman for his help with the reflectance determinations.

REFERENCES

(1) Burton, H. (1954). *J. Dairy Res.* **21**, 194.
(2) Burton, H. (1955). *J. Dairy Res.* **22**, 74.
(3) Hinton, C. L. & Macara, T. (1927). *Analyst*, **52**, 668.
(4) Rowland, S. J. (1938*a*). *J. Dairy Res.* **9**, 30.
(5) Rowland, S. J. (1938*b*). *J. Dairy Res.* **9**, 47.

(*MS. received for publication* 27 *July* 1954)

637.147.128

576. THE VOLATILE COMPOUNDS ASSOCIATED WITH OXIDIZED FLAVOUR IN SKIM MILK

By D. A. FORSS, E. G. PONT and W. STARK

Dairy Research Section, C.S.I.R.O., Melbourne, Australia

I. INTRODUCTION

Flavour defects in milk arising from oxidative reactions are of world-wide occurrence and have been extensively investigated. The literature dealing with this subject has been reviewed by Brown & Thurston (1), Greenbank (2), Kruisheer (3) and Lea (4). The off-flavours are usually described as cardboard, oily, metallic, tallowy and emery, and they are mostly referred to collectively as oxidized flavour. The defect is thought to be caused by oxidation of milk phospholipin (4, 5). This theory is supported by the weight of evidence, but it can be criticized on a number of grounds (6). Nothing is known definitely concerning the compounds responsible for the off-flavour, though there is some evidence to show that products of lipid oxidation are present. Pont (7), for instance, has reported that the defect was always accompanied by fat peroxide formation. Keeney & Doan (8) have found that neutral volatile fractions from oxidized butterfat when added to milk closely simulated the defect. Dunkley (9) has found that the thiobarbituric acid test for fat oxidation, when applied directly to milk, correlated well with the degree of oxidized flavour.

In a previous investigation from this laboratory Pont (10) observed that oxidized flavour caused by adding copper to milk was composite in character and that two main flavour components could be distinguished. One was an oily-metallic flavour caused by oxidation of the milk fat. The other component was described as cardboard; it came from the skim-milk constituents of the whole milk, since it could be readily induced in skim milk itself by the action of copper. This cardboard element in the flavour of the whole milk was usually the first to appear and it predominated in the early stages of the defect. The compounds responsible were steam volatile and could be distilled readily from whole or skim milk. Solvent extracts from the resultant distillates gave the cardboard odour in a highly concentrated form. The ease with which the flavouring substances could be isolated and concentrated in this way prompted an investigation into the nature and if possible the identity of the compounds responsible. The results of this investigation are given here.

II. EXPERIMENTAL

(*a*) *Treatment of skim milk for the production of cardboard flavour*

Skim milk of fat content below 0·06% by the butanol method was separated from good-quality raw market milk and pasteurized under commercial conditions at 73° C. for 15 sec. It was then held in a jacketed stainless steel vat in 100 gal. lots at 2° C. Copper in the form of $CuCl_2$ was added to give a Cu concentration of 5 p.p.m. At first 20 p.p.m. ascorbic acid was added also; this had been found previously to increase the intensity of off-flavour resulting from the action of copper (Pont (10)). Usually a well-developed cardboard flavour developed in 3 days and the milk was distilled after 4–7 days. Later in the investigation it

was found that even in milks which did not develop any cardboard flavour with the above treatment, the addition of 0·05 ml./l. of 30% H_2O_2 with the copper resulted in cardboard flavour. The peroxide treatment also intensified the cardboard flavour in normal milks.

(*b*) *Distillation of skim milk*

The milk was distilled, about 33 gal. at a time, in a jacketed stainless steel still at 5 mm. Hg pressure. The addition of 10 p.p.m. of 'Dow Corning Antifoam A' helped to control frothing. Steam heating was used, partly through the jacket and partly through a copper coil immersed in the milk. Some burning-on of the milk was encountered at the top of the froth level, though every effort was made to keep this to a minimum. In some of the later runs a totally submerged stainless-steel coil and on one occasion direct steam injection was used for heating; this eliminated the burning-on. The still was cleaned by boiling with a 2% NaOH solution after each run. Distillation was carried out at the rate of 3 gal./hr., and usually 6 gal./run (20%) was collected. Two stainless-steel condensers were used in series; the first was water-cooled and the second cooled with brine at 0° C. In previous laboratory-scale distillations examination of dry ice traps showed that there was a negligible loss of odour-producing material with simple condenser systems using ice-cooled water.

The distillates were held at 2° C. until the whole 100 gal. batch of milk had been distilled. They were then combined and redistilled in the same still, at first at atmospheric pressure but later under reduced pressure. 6 gal. of the secondary distillate were collected. The first 3 gal. which appeared to contain about 90% of the odoriferous material was extracted. The second 3 gal. was kept at 5° C. and added to the bulk distillate in the following week's run.

(*c*) *Treatment of control batches of skim milk*

Several batches of fresh skim milk free from cardboard flavour were distilled for control purposes. The control batches were of the same origin and were treated in the same way as the experimental batches except that the holding period was reduced to 2 days or less and copper was added immediately before distillation. In two control runs no copper was added; in one of these, the stainless-steel heating coil was used to obviate any copper contamination. No cardboard flavour was discernible in the milk of control runs.

(*d*) *Extraction of flavouring materials from distillate*

The flavouring compounds were extracted from the aqueous steam distillate with light petroleum; b.p. below 40° C. (In the early experiments the light petroleum was shaken with 20% oleum and then alkaline permanganate, giving a solvent which was almost pure pentane and transparent to ultra-violet light. When it was established that the cardboard compounds were aldehydes and/or ketones the light petroleum was treated only with 2N-HCl saturated with 2,4-dinitrophenylhydrazine (2,4-DNPH).) The 3 gal. steam distillate from 100 gal. skim milk was extracted with 5 × 1 l. lots of light petroleum, the extract dried over sodium sulphate overnight and the solvent removed through a 50 × 3 cm. fractionating column packed with Fenske helices. When all the solvent had been removed about 100 mg. of an oil with a strong cardboard odour was left. This was called the 'crude xt ract'.

(e) *Methods of isolation of compounds*

General information about the nature of the compounds responsible for the flavour was sought by preliminary chemical treatments of distillates and extracts. This work focused attention on the carbonyl compounds and means were sought to isolate and identify them. The method used for isolating the cardboard compounds from the 'crude extract' were based mainly on chromatographic separation, first of the free compounds and secondly of their derivatives.

Fractionation of free compounds

The free compounds were chromatographed on alumina, silica gel and celite, and silica-gel columns with light petroleum containing ethanol. Ether was used in one experiment, but in spite of all efforts to render it carbonyl-free, some contamination occurred; the use of this solvent was therefore discontinued. The odours of all fractions were appraised and those which appeared to contain cardboard compounds were converted to the 2,4-dinitrophenylhydrazones (2,4-DNPHones).

Fractionation of derivatives

p-Nitrophenylhydrazones, 2,4-DNPHones, semicarbazones and Girard P complexes were all chromatographed on paper and in columns. By far the most satisfactory derivatives for the separation and identification of carbonyl compounds were the 2, 4-DNPHones. They have the one disadvantage that the regeneration of the carbonyl compound is impracticable (11), so that determination of the flavour of the compounds isolated was not possible with this method.

2,4-*dinitrophenylhydrazones*

Preparation. Two main methods of preparing the 2,4-DNPHones were used. In one, the light petroleum extract of the aqueous distillate was added to a cold ethanol solution of excess 2,4-DNPH containing a few drops of concentrated HCl, and after 24 hr. the solvents were removed under reduced pressure. The residue was mixed with about 1 g. adsorbent and column chromatographed with carbonyl-free solvents.

In the other method the cardboard extract was added to 2 N-HCl saturated with 2,4-DNPH, and after shaking for 4–6 hr. the 2,4-DNPHones were extracted with carbonyl-free light petroleum. This extract was found to contain most of the cardboard compound 2,4-DNPHones.

Column chromatograms. Acid-washed alumina, silica gel and 'Celite 545', 2:1, and silica gel were used for chromatographic separation of these 2,4-DNPHones. It was essential to acid-wash the alumina as alkali reacts with these derivatives. With an alumina of Brockmann (12) activity of 2 the higher compounds moved slowly with light petroleum. When no further movement occurred with light petroleum, 0·1% ether was added and the concentration of ether doubled every 24 hr.

According to Gordon, Wopat, Burnham & Jones (13) a 2:1 mixture of silica gel and 'Celite 545, Johns-Manville' was the best adsorbent for the separation of 2,4-DNPHones, but later in our work, due to dissatisfaction with mixed adsorbents, the celite was dispensed with. The silica gel was prepared by the method of Fairbairn & Harpur (14). The

same solvent treatment as with alumina was used, but the 2,4-DNPHones would not move with light petroleum alone.

When the amount of 2,4-DNPHones to be separated was of the order of 1–20 mg., a column 55 × 2 cm. was used. For amounts less than this a column 30 × 1·5 cm. was used. A separation usually took 2–3 weeks.

While it was found that silica gel was better than alumina for the separation of compounds differing in molecular size, certain fractions from silica gel which contained compounds of about the same molecular size but differing in saturation could be separated on alumina.

(*f*) *Methods of identification of compounds*

The Girard reaction

Girard & Sandulesco (15) believed that only ketones were obtained from the decomposition of the Girard complex with mineral acids, but the work of Lederer & Nachmias (16) and Forss & Dunstone (17) has shown that aliphatic aldehyde Girard complexes can be decomposed with mineral acid. The Girard reaction thus proved effective only for the separation of carbonyl from non-carbonyl compounds, but was useful in the early establishment of the fact that the cardboard compounds were aldehydes and/or ketones. Attention was then concentrated on the 2,4-DNPHones.

Identification of 2,4-dinitrophenylhydrazones

Fractions of the mixed 2,4-DNPHones were rechromatographed until constancy of R_f, λ max. and m.p. was reached. In most cases the amount of pure compound obtained was below 1 mg., so microanalysis was impossible. Comparison of the compound with a synthetic compound thought to be the same, particularly by the method of mixed melting-points, was used to establish identity.

(1) *Paper chromatograms.* The paper chromatography of 2,4-DNPHones with methanol and light petroleum, b.p. 100–120° C., was based on the work of Huelin (18). With careful working the saturated aldehydes and ketones up to C_{10} and all unsaturated aldehydes encountered could be characterized by their R_f. When the λ max. of the spot was determined (see Braude & Jones (19)) a good conception of the molecular size and nature of the compound was obtained. The movements of 2,4-DNPHones on paper and silica gel corresponded in most cases, but in some there was a reversal of order.

(2) *Light absorption.* All light-absorption measurements were made on a 'Unicam S.P. 500, Spectrophotometer'. The 2,4-DNPHones were dissolved in 99% ethanol and the wave-length recorded in mμ. The λ max. of 2,4-DNPH was 351·5 mμ in both the 'Unicam' and in a 'Beckmann D.U. Spectrophotometer'.

III. RESULTS

(*a*) *Preliminary tests on distillate*

The cardboard odour in a steam distillate was destroyed by treatment with sodium bisulphite, potassium permanganate, semicarbazide hydrochloride, refluxing with N/1-KOH, 2,4-DNPH in N/1-HCl and sodium nitroprusside in N/1-NaOH. Treatment with 5% potassium bromate, refluxing with N/1-H_2SO_4, 4% mercuric chloride, Nessler's reagent and N/10-$AgNO_3$ decreased the odour. Changing the pH to 3 and 11 did not affect the odour.

From these tests it was tentatively concluded that the cardboard flavour was composed of aldehydes and/or ketones.

(*b*) *Preliminary tests on extracts*

Experiments on the 'crude extract'

Elemental analysis of the 'crude extract' by the sodium fusion method showed no nitrogen or halogen, but a trace of sulphur. No sulphur compounds however, were subsequently identified.

When the 'crude extract' was chromatographed on alumina with 1% ethanol in light petroleum, the cardboard compounds came through the column in a narrow band. This fraction, intensely cardboard in odour, consisted of about 25% of a red-brown oil and 75% of a white semi-crystalline mass. The cardboard odour was fairly stable; the fraction was held in the refrigerator for 6 months without noticeable loss of odour. At room temperature, the odour disappeared in a few weeks, especially from aqueous suspensions.

This cardboard fraction was easily soluble in light petroleum and chloroform, sparingly soluble in methanol and insoluble in water. When studied in the mass spectrograph it was found to be almost non-volatile in spite of its powerful odour, and did not give strong peaks. No aldehyde or sulphur peaks were observed and there was no parent peak. The infra-red spectrum indicated that NH_2^-, alcohol and aromatic groups were absent. There was evidence of several methyl groups and two carbonyl groups. The overall picture from the infra-red analysis was that a number of other compounds predominated over the carbonyl compounds present.

In distillation experiments it was found that most of the cardboard compounds distilled at below 70° C. at 0·01 mm. Hg pressure.

Experiments on the carbonyl fraction of the 'crude extract'

When a 'crude extract' was divided into two fractions by the Girard reaction both the carbonyl and the non-carbonyl fractions had strong odours, but only the former resembled cardboard. The λ max. in light petroleum of the carbonyl fraction was at 262 mμ, but after 10 days the main peaks were at 217, 223 and 227 mμ and there was a small peak at 255 mμ. (Martin, Schepartz & Daubert[20] found a λ max. of 262–3 mμ with reverted soybean oil. 2-enals or α, β-unsaturated aldehydes have a λ max. 220 ± 5 mμ.)

When the carbonyl fraction was added to skim milk the flavour was very similar to that of cardboard-flavoured skim milk. Results from infra-red examination of the carbonyl fraction were similar to those from the alumina cardboard fraction. Mass spectrographic examination did not yield any useful evidence.

(*c*) *Flavour tests on cardboard fractions*

At this stage it appeared that the compounds responsible for cardboard flavour were carbonyl in nature, were moderately volatile, and were unstable in air at room temperature. It was now thought desirable to test the efficiency of the isolation procedures used, and to compare the yields of cardboard compounds obtained by two techniques designed to separate carbonyl compounds. For this purpose the Girard and the metabisulphite reactions were most promising.

A test was arranged in which the intensities of cardboard flavour at different stages in the isolation procedure could be compared. Cardboard skim milk was distilled and the distillate extracted in the usual way with light petroleum. The light petroleum extract was divided into two fractions by distillation at 0·01 mm. up to 75° C. The volatile fraction

was in turn divided, one part being treated with the Girard reagent and another with concentrated metabisulphite solution. Samples from each step were added to fresh skim milk, and submitted to a grading panel for comparison of cardboard flavour. Owing to the lapse of time it was not possible to keep some of the original cardboard milk as a standard; another more recently prepared sample of cardboard skim milk was used for this purpose. Where passage through solvents was involved, solvents were tested for their effect on flavour by evaporating appropriate quantities and adding to skim milk in the same way. The results are shown in Table 1.

Table 1. *Relative intensities of cardboard flavour at consecutive stages in isolation from skim milk*

Fraction	Concentration in flavour tests	Intensity of cardboard flavour
A. Sample representing original milk		+ + + +
B. Steam distillate	As in original milk	+ +
C. Light petroleum extract of steam distillate, low boiling fraction	As in original milk	+ + + +
D. Light petroleum extract of steam distillate, high boiling fraction	9 times C	+
E. Bisulphite carbonyl fraction	45 times C	+ + + +
F. Bisulphite non-carbonyl fraction	7 times C	–
G. Girard carbonyl fraction	3 times C	+ + + +
H. Girard non-carbonyl fraction	15 times C	–

There is an anomaly in that the cardboard flavour intensity in the steam distillate was only half that of the volatile fraction later prepared from it. It is possible that the flavour in the distillate was masked by substances produced in the distillate and removed in subsequent procedures. However, it would appear that the isolation of the cardboard compounds was quantitative up to and including the volatile fraction of the 'crude extract'. About a two-thirds loss was then involved in the Girard process, and very little cardboard material was recoverable after the metabisulphite treatment for which regeneration is much less certain. It is presumed that cardboard compounds were destroyed by reaction with this reagent at a double bond.

(*d*) *Control experiments*

It was thought desirable to investigate the occurrence of carbonyl compounds in skim milk free of cardboard flavour, since only the additional ones present in cardboard milk would be of significance.

Steam distillates from an all-glass laboratory still of skim milk, skim milk with copper added just before distillation ('copper distillate'), and the same milk in which the cardboard flavour had been developed by treating with copper and ascorbic acid for 5 days, all had a similar type of odour, but the latter two were more intense. When the distillates were added to fresh skim milk, only the distillate from cardboard milk produced the cardboard flavour. Study of the 2,4-DNPH derivatives revealed that* acetone was the major carbonyl compound of all three distillates. This indicated that the cardboard compounds represent only a small part of the total carbonyl compounds in the distillate. The acetone

* Since this paper was written, it has been observed that when acetone and *n*-hexanal 2,4-DNPHones were chromatographed on silica columns with ether and light petroleum, they underwent partial decomposition to a yellow compound, m.p. 141–3° C., λ max. 353 mμ and R_f equal to acetaldehyde. The finding would not appear to invalidate any of the important conclusions reported in the present paper.

was an embarrassment when the steam distillate was treated directly with 2,4-DNPH and HCl and the 2.4-DNPHones extracted with light petroleum. When the steam distillate was extracted with light petroleum, most of the acetone remained in the aqueous solution.

When copper was added to 100 gal. skim milk just before it was distilled in the apparatus with a copper coil, the odour of the steam distillate resembled that from a cardboard run, but when added to skim milk the flavour was merely burnt and rubbery. The 'crude extract' which only had a slight cardboard odour was treated with 2,4-DNPH and HCl in ethanol. The only compounds revealed on alumina and paper analysis were a small amount of saturated material in the methyl propyl to methyl amyl ketone region. The 2,4-DNPHones obtained from the steam distillate, after it had been extracted with light petroleum, were similar to those obtained by the same procedure from a normal cardboard run. On alumina analysis after the acetone and acetaldehyde had been eluted, a compound (3·4 mg.) with the following properties was obtained: deep red crystals from ethanol, m.p. 157–60° C., only sparingly soluble in ethanol, R_f just below acetaldehyde and λmax. 358 mμ (Found: C, 46·3; H, 3·8%).

When 100 gal. skim milk was distilled with only a stainless-steel coil about 1 mg. of 2,4-DNPHones was obtained. Most of this fraction had an R_f equal to acetone and λmax. 358 mμ.

(e) *Compounds isolated and identified*

Silica-gel analysis of cardboard compounds

Fractionation of the 'crude extract' on silica gel did not result in the separation of pure compounds. Fractionation of the 2,4-DNPHones derived from the cardboard fractions from silica gel resulted, however, in the isolation of several 2-enals and probably 2,4-dienals with R_f's methyl ethyl ketone to methyl nonyl ketone. Details of the identification of these compounds are recorded in Table 2 and the following section.

Other 2,4-*dinitrophenylhydrazones isolated from cardboard skim milk*

(i) Orange crystals, m.p. 107–9° C. from ethanol, R_f above 2-undecenal, λmax. 375 mμ. Found: C, 61·3; H, 7·7%, Calc. for $C_{20}H_{30}N_4O_4$ – C, 61·5; H, 7·7%. This is possibly the C_{14} 2-enal.

(ii) Deep red crystals, m.p. 153–5° C. from light petroleum, R_f just below methyl ethyl ketone, λmax. 389 mμ.

(iii) Deep red crystals, m.p. 130° C. from light petroleum, R_f equal to methyl propyl ketone, λmax. 391 mμ.

(iv) Deep red crystals, m.p. 130–5° C. from light petroleum, R_f equal to methyl butyl ketone, λmax. 390 mμ.

Comparing (ii), (iii) and (iv) with 2,4-hexadienal, deep red crystals, m.p. 186–7° C. from ethanol, R_f between acetaldehyde and acetone and λmax. 389 mμ, it seems that these three compounds are 2,4-dienal derivatives with more carbons than 6. Probably one of these compounds is the 2,4-decadienal 2,4-DNPHone of Stapf & Daubert (23) and Swift, O'Connor, Brown & Dollear (24).

Preparation of aldehydes for reference standards

n-*Hexanal.* This compound was prepared by the dry distillation of calcium caproate and calcium formate. 2,4-DNPHone, m.p. 102–4° C. (Brady & Elsmie (25), m.p. 104° C.). Found: C, 52·2; H, 6·0; O, 23·0%. Calc. for $C_{12}H_{16}N_4O_4$: C, 51·4; H, 5·7; O, 22·9%.

Table 2. *Identification of the 2,4-dinitrophenylhydrazones isolated from cardboard skim milk*

2,4-Dinitrophenylhydrazone	Colour	M.p., solvent in brackets, LP = light petroleum	Mixed m.p. with authentic sample	M.p. of melt of mixed m.p. from column 4	R_f compound with equivalent value	λ max. (mμ)
Isolated acetaldehyde	Yellow	144–8° C. (EtOH)	—	—	Acetaldehyde	357
		156–8° C. (LP)*				
Synthetic acetaldehyde	Yellow	163° C.	—	—	—	357
Isolated *n*-hexanal	Yellow	99–101° C. (LP)	99–101° C.	—	*n*-Hexanal	358
Synthetic *n*-hexanal	Yellow	102–4° C. (EtOH)	—	—	—	358
Isolated crotonaldehyde	Red	190° C. (EtOH)	Authentic sample unavailable when isolated	—	Crotonaldehyde	373
Synthetic crotonaldehyde	Red	190° C. (EtOH)		—	—	373
Isolated 2-pentenal	Orange	155–7° C. (LP)	154–7° C.	154–6° C.	2-Pentenal	374
Synthetic 2-pentenal	Orange	154–6° C. (EtOH)		—	—	374
Isolated 2-hexenal	Orange	141–2° C. (LP)	141–4° C.	143–4° C.	2-Hexenal	373
Synthetic 2-hexenal	Orange	143–4° C. (EtOH)	—	—	—	374
Isolated 2-heptenal	Orange	122–4° C. (LP)	122–4° C.	124–5° C.	2-Heptenal	374
Synthetic 2-heptenal	Orange	123–5° C. (LP)	—	—	—	374
Isolated 2-octenal	Orange	126–7° C. (LP)	127–8° C.	126–7° C.	2-Octenal	375
Synthetic 2-octenal	Orange	126–8° C. (EtOH)	—	—	—	375
Isolated 2-nonenal	Orange	119–22° C. (LP)	119–22° C.	122–4° C.	2-Nonenal	375
Synthetic 2-nonenal	Orange	122–4° C. (EtOH)	—	—	—	375
Isolated 2-decenal	Orange	123–5° C. (LP)	123–5° C.	122–4° C.	2-Decenal	375
Synthetic 2-decenal	Orange	124–6° C. (EtOH)	—	—	—	375
Isolated 2-undecenal	Orange	119–20° C. (LP)	119–20° C.	119–20° C.	2-Undecenal	375
Synthetic 2-undecenal	Orange	119–20° C. (EtOH)	—	—	—	375

* The 2,4-dinitrophenylhydrazone of acetaldehyde has a number of melting-points—see Braddock *et al.* (31) and Huntress & Mulliken (29).

2-*Pentenal.* 2-Pentenal was prepared by the method of Kuhn & Grundmann (26). 2,4-DNPHone, m.p. 154–6° C. (Kawahara & Dutton (27), m.p. 162–3° C.). Found: C, 50·0; H, 4·3; O, 23·9%. Calc. for $C_{11}H_{12}N_4O_4$: C, 50·0; H, 4·5; O, 24·3%. Semicarbazone: m.p. 166–9° C. (Gouge (28), m.p. 179° C.).

2-*Hexenal.* The preparation of 2-hexenal was based on the method of Kuhn & Grundmann. 2,4-DNPHone, m.p. 143–4° C. (Takei, Sakato & Kuroiwa (29), m.p. 144° C.). Found: C, 51·9; H, 4·9; N, 20·8%. Calc. for $C_{12}H_{14}N_4O_4$: C, 51·8; H, 5·0; N, 20·2%. Semicarbazone: m.p. 162° C. (Takei *et al* (29), m.p. 173° C.).

2-*Octenal.* The preparation of 2-octenal was also based on the method of Kuhn & Grundmann. 2,4-DNPHone, m.p. 126–8° C. Found: C, 55·0; H, 5·5; N, 18·7%. Calc. for $C_{14}H_{18}N_4O_4$: C, 54·9; H, 5·9; N, 18·3%. Semicarbazone: m.p. 163–4° C.

2-*Nonenal.* 2-Nonenal was prepared from castor oil by the method of Scanlan & Swern (31). 2,4-DNPHone, m.p. 122–4° C. (Martin, Schepartz & Daubert (30), m.p. 124·4–5° C.). Found: C, 56·3; H, 6·1; N, 17·8; O, 19·4%. Calc. for $C_{15}H_{20}N_4O_4$: C, 56·2; H, 6·3; N, 17·5; O, 20·0%. Semicarbazone from methanol: m.p. 164–6° C. (Martin *et al.* (30), m.p. 164–5° C.).

2,4-*Hexadienal.* 2, 4-Hexadienal was prepared according to the method of Kuhn & Hoffer (32) by condensing CH_3CHO and $CH_3CH{:}CH.CHO$ in the presence of piperidine. 2,4-DNPHone. Found: C, 52·1; H, 4·2; N, 19·9; O, 22·8%. Calc. for $C_{12}H_{12}N_4O_4$: C, 52·2; H, 4·3; N, 20·3; O, 23·2%. Semicarbazone: m.p. 201–2° C. (Baumgarten & Glatzel (33), m.p. 203° C.), λmax. 292 mμ in ethanol.

Carbonyl compounds remaining in steam distillate after light petroleum extraction

The 3 gal. extracted steam distillate was treated with 1 g. 2,4-DNPH and 25 ml. conc. HCl for 3 days and the yellow solution extracted with light petroleum. After removal of the solvent about 200 mg. of a mixture of yellow and orange crystals was obtained.

Paper chromatogram:

R_f	λ max. (mμ)
Acetaldehyde	358
Between acetaldehyde and acetone	359
Acetone (95%)	359

The material was crystallized to constant m.p. 125° C., acetone m.p. 125–6° C., mixed m.p. 125–6° C. Found: C, 46·3, 46·5; H, 4·6, 4·4; N, 23·3; O, 26·5%. Calc. for $C_9H_{10}N_4O_4$: C, 45·4; H, 4·2; N, 23·5; O, 26·9%.

A control run with 3 gal. distilled water yielded less than 1 mg. of a yellow material with R_f almost 0.

When this experiment was repeated with only 2 ml. HCl a similar result was obtained.

These two experiments showed that only acetone and an acetaldehyde-like compound were left in the steam distillate after light petroleum extraction.

(*f*) *Flavour tests on pure compounds*

To establish whether any of the compounds isolated were contributing to the cardboard flavour, it was necessary to study their effect on the flavour of skim milk.

Acetaldehyde, acetone, crotonaldehyde, *n*-hexanal, 2-pentenal and 2-hexenal when added to skim milk did not produce flavours resembling cardboard.

2-Nonenal prepared from 9,10,12-trihydroxystearic acid had a flavour identical with that of 2-nonenal from its semicarbazone treated with dilute sulphuric acid. 2-Octenal

was prepared from its semicarbazone. They produced a flavour closely resembling if not identical with cardboard flavour. The odour, however, was not as strong as that associated with this defect.

Although 2,4-dienals had not been identified from cardboard skim milk, several compounds most probably of this type were isolated. 2,4-Hexadienal was the only compound in this group the preparation of which was described in the literature. It was thought that its flavour would resemble that of the higher dienes that we have isolated. The flavour produced by the addition of 2,4-hexadienal to skim milk was also considered by the tasting panel to resemble closely the cardboard flavour. The flavour was different from that of the 2-enals and it was remarked that it masked all trace of the natural milk flavour.

IV. DISCUSSION AND CONCLUSIONS

The compounds identified as constituents of distillates from cardboard-flavoured skim milk are acetone, acetaldehyde, *n*-hexanal, crotonaldehyde, 2-pentenal, 2-hexenal, 2-heptenal, 2-octenal, 2-nonenal, 2-decenal and 2-undecenal. Of these acetaldehyde and acetone can be eliminated as possible causes of cardboard flavour as they were found in normal skim milk. There is presumptive evidence also for the occurrence of several 2,4-dienals of short to medium chain length, but without reference standards due to the difficulty of synthesizing these compounds their identities could not be confirmed.

The C_6 to C_{11} 2-unsaturated aldehydes are the most significant from the flavour-determining viewpoint. In concentrated form these compounds have an intense odour resembling that of drying oil or paint. They were not found in distillates from control unoxidized milk; assuming full recovery, their concentration in cardboard-flavoured skim milk would be of the order of 1 part in 10^7 to 10^9. When added to fresh skim milk in this range of concentration, they impart a flavour which closely resembles the cardboard defect. It is concluded, therefore, that this group of unsaturated aldehydes is a basic element in copper-induced cardboard flavour in milk.

There is some evidence to suggest that the dienals may also be a highly significant element in cardboard flavour. Only one compound of this type (2,4-hexadienal) was available for flavour testing in milk. It imparted a flavour which also closely resembled the cardboard flavour compounds tested.

While *n*-hexanal, crotonaldehyde and 2-pentenal do not, in themselves, reproduce cardboard flavour when added to milk, the nature of the flavour they impart suggests that they could play a subsidiary role in the blend of flavours constituting the defect.

The knowledge of the volatile compounds associated with cardboard flavour in milk gained by this investigation provides some further clues to the origin of the defect. α, β-unsaturated carbonyl compounds of the type found here are believed to be characteristic breakdown products of oxidizing fat and to contribute substantially to resultant off-flavours. Their occurrence in butter, paralleling the development of oily flavour, has been reported by Holm, Wode & Thomé (34) and Thomé & Mattsson (35). In addition, 2-heptenal has been isolated by Schepartz & Daubert (36) from reverted hydrogenated soybean oil, 2-octenal from oxidized cottonseed oil by Swift *et al.* (24) and 2-undecenal from methyl hydroperoxido oleate by Swift, Dollear, Brown & O'Connor (37).

The association of this type of compound with cardboard flavour in milk is further evidence that the defect results from some form of lipid oxidation. Furthermore, the unsaturated character of the C_4 to C_{11} mono-enals and the presumed 2,4-dienals points to

their derivation from the oxidation of dienoic and more highly unsaturated fatty acids. The greatly increased susceptibility to autoxidation of fatty acids with increasing unsaturation is well known; this is consistent with the observation that cardboard flavour is usually the first component of oxidized flavour to appear in whole milk and predominates in the early stages of the defect (10).

V. SUMMARY

Using chromatographic techniques, a number of compounds was isolated from the steam distillates of skim milk containing oxidized (cardboard) flavour. The following compounds were definitely identified: acetone, acetaldehyde, *n*-hexanal, crotonaldehyde, and the C_5 to C_{11} 2-unsaturated aldehydes. Presumptive evidence for the presence of several 2,4-diunsaturated aldehydes of medium chain length was obtained also. Acetaldehyde and acetone were also isolated from normal skim milk; these two compounds appear to play no part in the defect.

2-Enals, in particular the C_8 and C_9, were considered to be the principal flavour-determining compounds. In milk at dilutions of 1 part in 10^7 to 10^9 they impart an oxidized type of flavour resembling that known as cardboard. Evidence was obtained that 2,4-dienals were also present and they produced a similar flavour.

It may further be deduced that cardboard flavour in milk is caused by the preferential oxidation of di- and polyunsaturated fatty acids.

The authors wish to express their gratitude for assistance from the following: the School of Dairy Technology, Werribee, and 'Model Dairy Ltd.', Kew, both of Victoria, for skim milk; Mr B. W. Wilson for preparing the apparatus for steam distillations; Mr R. L. Werner, New South Wales University of Technology, for infra-red analysis; Dr K. W. Zimmerman, C.S.I.R.O. Microanalytic Laboratory, for microanalyses; Dr A. I. Schepartz, Veterans Administration Hospital, Pennsylvania, U.S.A., for a sample of the 2,4-DNPHone of 2-heptenal; Dr R. R. Allen, of Armour and Co., Chicago, U.S.A., for samples of the 2,4-DNPHones of 2-decenal and 2-undecenal; Mr W. G. Laver, who assisted for 3 months; Mr E. A. Dunstone for technical assistance, and finally Mr G. Loftus Hills for helpful advice.

The investigations here reported have been carried out as part of the research programme of the Dairy Research Section of the Australian Commonwealth Scientific and Industrial Research Organization.

REFERENCES

(1) Brown, W. C. & Thurston, L. M. (1940). *J. Dairy Sci.* **23**, 629.
(2) Greenbank, G. R. (1948). *J. Dairy Sci.* **31**, 913.
(3) Kruisheer, C. I. (1952). *J. Neth. Milk & Dairy*, **6**, 242.
(4) Lea, C. H. (1953). *XIIIth Int. Dairy Congr.* **3**, sect. IV, 1037.
(5) Greenbank, G. R. (1949). *XIIth Int. Dairy Congr.*, **2**, sect. II, p. 284.
(6) Pont, E. G. (1953). *XIIIth Int. Dairy Congr.* **4**, sect. IV, p. 320.
(7) Pont, E. G. (1953). *XIIIth Int. Dairy Congr.* **3**, sect. IV, p. 1049.
(8) Keeney, M. & Doan, F. J. (1951). *J. Dairy Sci.* **34**, 713.
(9) Dunkley, W. L. (1951). *Food Technol.* **5**, 342.
(10) Pont, E. G. (1952). *J. Dairy Res.* **19**, 316.
(11) Demaecker, J. & Martin, R. H. (1954). *Nature, Lond.*, **173**, 266.
(12) Brockmann, H. & Schodder, H. (1941). *Ber. dtsch. chem. Ges.* **74**, 73.

(13) Gordon, B. E., Wopat, Jr., F., Burnham, H. D. & Jones, Jr., L. C. (1951). *Anal. Chem.* **23**, 1754.
(14) Fairbairn, D. & Harpur, R. P. (1951). *Canad. J. Chem.* **29**, 633.
(15) Girard, A. & Sandulesco, G. (1936). *Helv. chim. acta.* **19**, 1095.
(16) Lederer, E. & Nachmias, G. (1949). *Bull. soc. chim. Fr.* 400.
(17) Forss, D. A. & Dunstone, E. A. (1954). *Nature, Lond.*, **173**, 401.
(18) Huelin, F. E. (1952). *Austr. J. sci. Res.* B, **5**, 328.
(19) Braude, E. A. & Jones, E. R. H. (1945). *J. chem. Soc.* p. 498.
(20) Martin, C. J., Schepartz, A. I. & Daubert, B. F. (1948). *J. Amer. Oil Chem. Soc.* **25**, 113.
(21) Braddock, L. I., Garlow, K. Y., Grim, L. I., Kirkpatrick, A. F., Pease, S. W., Pollard, A. J., Price, E. F., Reissmann, T. L., Rose, H. A. & Willard, M. L. (1953). *Anal. Chem.* **25**, 301.
(22) Huntress, E. H. & Mulliken, S. P. (1946). *Identification of Pure Organic Compounds*, Order I, p. 43. New York: John Wiley and Sons, Inc.
(23) Stapf, R. J. & Daubert, B. F. (1950). *J. Amer. Oil Chem. Soc.* **27**, 376.
(24) Swift, C. E., O'Connor, R. T., Brown, L. E. & Dollear, F. G. (1949). *J. Amer. Oil Chem. Soc.* **26**, 297.
(25) Brady, O. L. & Elsmie, G. V. (1926). *Analyst*, **51**, 77.
(26) Kuhn, R. & Grundmann, M. (1937). *Ber. dtsch. chem. Ges.* **70**, 1897.
(27) Kawahara, F. K. & Dutton, H. J. (1952). *J. Amer. Oil Chem. Soc.* **29**, 372.
(28) Gouge, M. (1951). *Ann. chim.* **6**, 648.
(29) Takei, S., Sakato, G., Ono, M. & Kuroiwa, Y. (1938). *J. Agric. chem. Soc. Japan*, **14**, 709.
(30) Martin, C. J., Schepartz, A. I. & Daubert, B. F. (1948). *J. Amer. chem. Soc.* **70**, 2601.
(31) Scanlan, J. T. & Swern, D. (1940). *J. Amer. chem. Soc.* **62**, 2309.
(32) Kuhn, R. & Hoffer, M. (1930). *Ber. dtsch. chem. Ges.* **63**, 2164.
(33) Baumgarten, P. & Glatzel, G. (1926). *Ber. dtsch. chem. Ges.* **59**B, 2664.
(34) Holm, U., Wode, G. & Thomé, K. E. (1952). *Medd. Statens Mejeriförsök*, no. 37.
(35) Thomé, K. E. & Mattsson, S. (1953). *XIIIth Int. Dairy Congr.* **3**, sect. iv, p. 1056.
(36) Schepartz, A. I. & Daubert, B. F. (1950). *J. Amer. Oil Chem. Soc.* **27**, 367.
(37) Swift, C. E., Dollear, F. G., Brown, L. E. & O'Connor, R. T. (1948). *J. Amer. Oil Chem. Soc.* **25**, 39.

(*MS. received for publication* 3 *August* 1954)

637.17:636.4:54

577. THE CHEMICAL COMPOSITION OF THE COLOSTRUM AND MILK OF THE SOW

By DAWN R. PERRIN

Ruakura Animal Research Station, New Zealand Department of Agriculture

Studies reported in an earlier paper (1) indicated that there is a rapid change in the percentages of the major constituents of sow's milk during the week following parturition. This paper records a more detailed investigation of the colostrum and milk from four sows over lactation periods of 8 weeks. Analyses for mineral constituents were also undertaken on samples obtained throughout the lactation of two sows.

EXPERIMENTAL

The sows studied were of the Berkshire breed. Sow A was in her fourth, and sows B, C and D in their third lactation.

During parturition colostrum samples were obtained readily by hand milking. When the sow began to control let-down, usually about 2–3 hr. after the onset of parturition, pitocin injections were used and the milk was withdrawn by hand or by milking machine. From 15 hr. after parturition all samples were obtained by machine milking. The colostrum samples were obtained for the most part at 3-hourly intervals up to the thirtieth hour, then at 12 or 24 hr. intervals for the following 2 days. Thereafter samples were taken once a week for the rest of an 8-week lactation.

ANALYTICAL METHODS

The methods used for the determination of total solids, fat, protein, lactose and ash have already been described (1). For colostrum, smaller portions were used for the total solids and protein analyses. For some early samples which were of small volume, the fat determination was done on an accurately weighed small amount, usually 4–6 g.

The chloride content was determined on an aliquot of the filtrate prepared for the Luff-Schoorl estimation of lactose by direct titration with standard mercuric nitrate in the presence of diphenyl carbazone as indicator.

For the remaining mineral constitutents, the ash (from 10 g. milk) was dissolved in 20 ml. of approximately N/5-hydrochloric acid and diluted to 100 ml. A 10 ml. portion of this acid solution was transferred to a 20 ml. volumetric flask and neutralized to methyl red using ammonia solution.

Calcium was estimated on a 2 ml. aliquot by the procedure described by Clark-Collip (2) for blood serum.

Magnesium was precipitated as magnesium ammonium phosphate from an aliquot of the supernatant fluid from the calcium determination, and after washing was determined as phosphate (3).

Total phosphorus was determined by the method of Fiske and Subba Row for blood serum, using 1.2.4 amino-naphtholsulphonic acid as reducing agent (4).

RESULTS AND DISCUSSION

The results for the major constituents of colostrum and milk of four sows are given in Table 1. The initial secretions were characterized by high protein and low fat and lactose values. All sows showed similar trends in composition from colostrum at parturition through the transition to milk. These changes appeared to take place at slightly different rates for the various constituents, and thereafter any particular constituents altered much more slowly as lactation became established. Thus after a rapid decrease, the protein content became more or less steady at a time when the lactose and ash were still increasing.

Table 1. *Effect of stage of lactation on major constituents of sow's colostrum and milk (expressed as percentage by weight)*

	Sow A						Sow B					
Time in milk	Total solids	Fat	Solids-not-fat	Protein	Lactose	Ash	Total solids	Fat	Solids-not-fat	Protein	Lactose	Ash
At parturition	29·41	7·00	22·41	17·18	2·48	0·66	33·10	7·62	25·48	22·55	1·97	0·63
After: 3 hr.	29·05	7·66	21·39	16·78	2·49	0·64	31·35	7·81	23·64	20·57	2·20	0·62
6 hr.	26·45	8·39	18·06	14·55	2·82	0·64	29·19	8·59	20·60	17·33	2·46	0·62
9 hr.	21·42	6·66	14·76	10·27	3·09	—	25·84	8·90	16·94	13·02	2·87	0·63
12 hr.	20·81	7·84	12·97	8·59	3·09	0·65	22·29	8·28	14·01	9·81	3·34	0·63
15 hr.	19·27	7·30	12·20	7·07	3·11	0·65	19·39	7·40	11·99	7·77	3·62	0·63
18 hr.	19·14	7·55	11·59	6·96	3·12	0·71	19·58	7·45	12·13	7·21	3·96	0·63
21 hr.	19·39	7·60	11·79	6·93	3·25	0·72	19·55	8·30	11·25	6·75	3·88	0·64
24 hr.	21·76	10·70	11·06	6·91	3·27	0·72	21·62	9·17	12·45	8·12	3·67	0·64
27 hr.	20·37	8·75	11·62	6·75	3·47	0·79	22·48	11·55	10·93	6·40	3·74	0·64
30 hr.	21·63	10·20	11·43	7·11	3·34	0·79	21·59	10·45	11·14	6·36	3·90	0·66
33–48 hr.	20·29	8·60	11·69	7·06	3·91	0·81	—	—	—	—	—	—
3rd day	28·35	17·20	11·15	6·87	3·62	0·77	20·36	9·05	11·31	6·17	4·40	0·77
4th day	21·52	9·85	11·67	6·44	4·33	0·83	—	—	—	—	—	—
5th day	—	—	—	—	—	—	22·11	10·90	11·21	5·92	4·62	0·78
2nd week	22·49	11·70	10·97	5·55	4·55	0·84	21·33	10·10	11·23	5·05	5·20	0·74
3rd week	21·21	10·05	11·16	5·24	5·00	0·80	19·94	10·10	9·84	4·51	4·33	0·71
4th week	22·45	11·15	11·30	5·48	4·90	0·86	20·42	9·90	10·52	5·04	4·73	0·77
5th week	21·12	9·45	11·67	6·00	4·81	0·89	22·94	11·58	11·36	5·99	4·56	0·90
6th week	20·54	8·20	12·34	6·34	5·11	1·03	23·17	11·40	11·77	6·31	4·26	0·98
7th week	20·48	8·15	12·33	6·39	4·95	1·08	21·89	9·35	12·54	7·16	4·14	1·04
8th week	22·25	8·60	13·65	8·18	4·31	1·19	24·99	10·50	14·49	9·72	3·59	1·16

	Sow C						Sow D					
Time in milk	Total solids	Fat	Solids-not-fat	Protein	Lactose	Ash	Total solids	Fat	Solids-not-fat	Protein	Lactose	Ash
At parturition	30·73	7·70	23·03	18·84	2·45	0·62	27·60	6·51	21·09	16·96	3·09	0·62
After: 3 hr.	28·94	7·75	21·19	17·17	2·72	0·62	25·36	5·87	19·49	15·53	3·28	0·58
6 hr.	—	—	—	—	—	—	24·17	6·35	17·32	13·68	3·31	0·60
9 hr.	—	—	—	—	—	—	—	—	—	—	—	—
12 hr.	—	—	—	—	—	—	19·18	5·54	13·64	9·07	3·87	0·62
15 hr.	19·13	7·45	11·68	6·94	3·72	0·67	—	—	—	—	—	—
18 hr.	—	—	—	—	—	—	18·69	5·77	12·92	7·42	4·49	0·64
21 hr.	—	—	—	—	—	—	—	—	—	—	—	—
24 hr.	—	—	—	—	—	—	18·15	6·28	11·87	6·81	4·63	0·65
27 hr.	—	—	—	—	—	—	—	—	—	—	—	—
30 hr.	—	—	—	—	—	—	19·70	7·33	12·37	7·41	4·54	0·65
33–48 hr.	20·59	8·75	11·84	6·52	4·18	0·76	22·48	10·10	12·38	7·39	4·58	0·68
3rd day	21·01	10·05	10·96	5·58	4·38	0·74	23·94	11·75	12·19	6·59	4·65	0·71
4th day	18·44	7·30	11·14	5·37	4·86	0·80	21·89	10·75	11·14	5·89	4·99	0·73
5th day	19·75	8·20	11·55	5·58	5·18	0·80	20·41	9·10	11·31	5·27	5·33	0·79
2nd week	21·49	9·90	11·59	5·65	5·31	—	22·82	11·55	11·27	5·35	5·24	—
3rd week	20·31	9·30	11·01	5·26	5·74	—	19·77	9·20	10·57	4·87	5·12	—
4th week	20·81	9·30	11·51	5·38	5·26	—	19·77	8·70	11·07	5·52	4·70	0·84
5th week	21·89	10·25	11·64	6·72	4·49	—	19·06	7·30	11·76	6·10	5·07	0·89
6th week	20·46	7·90	12·56	6·77	4·81	—	19·36	7·40	11·96	6·22	4·97	—
7th week	19·92	7·15	12·77	7·07	4·87	—	20·72	7·50	13·22	7·52	4·37	1·16
8th week	20·78	7·70	13·08	7·26	4·81	1·07	19·93	6·45	13·48	7·83	4·52	1·16

Average values during this transition period from parturition onwards were as follows:

(1) Protein falling from 18·8 to 7·1% after 24 hr.

(2) Lactose rising from 2·4 to 4·4% after 3 days.

(3) Fat rising from 7·2 to 12·5% after 48 hr., to fall again to 8·8% at the fourth or fifth day.

(4) Ash rising from 0·63 to 0·80% after 5 days.

The steadily decreasing protein and increasing fat and lactose in the colostrum of the sow is similar to that reported for other species. The observation that the colostral fat rises initially to a high value and then drops to a value approaching that of normal milk is similar to that reported by Elsdon for the dairy cow (5).

Over the remainder of the lactation, changes in all major constituents were similar to those previously reported (1).

The ash content of sow's colostrum rises steadily from parturition onwards for about 5 days, and then increases more gradually over the course of the lactation. In this increase in mineral content after parturition, sow's colostrum differs from that recorded for most other species. In the transition from colostrum to milk in cows, goats, ewes, mares and women, there is a decrease in ash content as lactation becomes established.

These results would suggest that the transition from colostrum to milk takes place within about 3 days. However, in this study, when early samples for analysis were taken 3-hourly, in addition to normal suckling by the litter, it is likely that a considerably increased amount of colostrum or transition fluid was withdrawn during the first day or so. Such frequency of sampling may increase the actual rate of change, but it is considered that analyses of such material would still show any changes in composition in the order in which they would normally occur. From earlier results at Ruakura, when the first milk sample was taken between the fourth and sixth days, it appeared likely that the sow's milk is not of normal composition until the end of the first week. This has been noticed particularly in respect to first litter sows.

It is also interesting to note that on two occasions (different sows) when the milk was withdrawn included in error fluid from unnursed teats, the analysis of the composite material gave values intermediate between a colostrum at parturition and fluid from the nursed teats at approximately the same stage post-partum.

The mineral constituents of colostrum and milk

The composition of the ash of colostrum and milk throughout the lactation is given in Table 2.

The main trends were:

(1) A four-fold increase in calcium content from colostrum at parturition to normal milk at 8 days, followed by a more gradual increase over the rest of the lactation.

(2) A less marked increase in phosphorus throughout the lactation.

(3) A slight decline in magnesium over the first day with subsequently a slow rise over the lactation.

(4) An increase in chloride over the first 2–3 days, and thereafter a gradual decrease until the end of the lactation.

All samples of sow's colostrum up to 30 hr. after parturition contained more phosphorus than calcium, and the Ca/P ratio showed a steady increase with advancing lactation. The

magnesium content of sow's colostrum and milk is lower than is reported for the milk of other species.

Calculation of the calcium, magnesium and phosphorus as the sum of the oxides and chloride as the sodium or potassium salt, give for most of the analyses a value fairly close to the total ash as determined directly. It seems likely therefore that these constituents account for most of the ash.

Table 2. *Effect of stage of lactation on mineral constituents of sow's colostrum and milk (expressed as percentage by weight)*

	Sow A					Sow B				
Time in milk	Ash	Ca	Mg	P	Cl	Ash	Ca	Mg	P	Cl
At parturition	0·66	0·055	0·018	0·111	0·097	0·63	—	—	—	0·091
After: 3 hr.	0·64	0·049	0·013	0·111	0·103	0·62	0·041	0·013	0·108	0·091
6 hr.	0·64	0·054	0·015	0·114	0·121	0·62	0·042	0·010	0·107	0·103
9 hr.	—	—	—	—	—	0·63	0·052	0·011	0·108	0·103
12 hr.	0·65	0·068	0·013	0·113	0·127	0·63	0·055	0·011	0·107	0·107
15 hr.	0·65	0·075	0·014	0·115	0·127	0·63	0·060	0·010	0·109	0·115
18 hr.	0·71	0·078	0·009	0·120	0·121	0·63	0·066	0·012	0·116	0·103
21 hr.	0·72	0·081	0·009	0·115	0·115	0·64	0·066	0·010	0·113	—
24 hr.	0·72	0·087	0·009	0·125	0·103	0·64	0·076	0·010	0·115	0·121
27 hr.	0·79	0·107	0·009	0·121	—	0·64	0·079	0·009	0·121	0·121
30 hr.	0·79	0·122	0·010	0·145	0·121	0·66	0·104	0·009	0·127	—
33 hr.	0·81	0·143	0·010	0·141	0·121	0·65	0·087	0·011	0·103	0·097
42 hr.	—	—	—	—	—	0·69	0·105	0·011	0·114	0·115
48 hr.	0·77	0·168	0·011	0·144	0·103	—	—	—	—	—
55 hr.	—	—	—	—	—	0·77	0·147	0·011	0·135	0·103
72 hr.	0·83	0·182	0·012	0·142	0·134	—	—	—	—	—
80 hr.	—	—	—	—	—	0·78	0·170	0·012	0·140	0·115
8 days	—	—	—	—	—	0·74	0·172	0·013	0·130	0·127
9 days	0·84	0·199	0·010	0·127	0·115	—	—	—	—	—
16 days	0·80	0·214	0·012	0·139	0·097	0·71	0·184	0·012	0·120	0·121
21 days	—	—	—	—	—	0·77	0·201	0·014	0·132	0·115
23 days	0·86	0·233	0·013	0·148	0·091	—	—	—	—	—
28 days	—	—	—	—	—	0·90	0·255	0·015	0·143	0·121
30 days	0·89	0·268	0·011	0·155	0·097	—	—	—	—	—
33 days	—	—	—	—	—	0·89	0·258	0·018	0·151	0·097
37 days	1·03	0·288	0·012	0·178	0·091	—	—	—	—	—
40 days	—	—	—	—	—	0·98	0·273	0·017	0·154	0·097
45 days	1·08	0·317	0·015	0·196	0·091	—	—	—	—	—
47 days	—	—	—	—	—	1·04	0·279	0·014	0·156	0·091
50 days	1·19	0·343	0·017	0·212	0·091	—	—	—	—	—
54 days	—	—	—	—	—	1·16	0·324	0·018	0·175	0·097

Comparison with results previously reported

Studies on the composition of sow colostrum have been reported by Hughes & Hart (6) and Braude, Coates Henry, Kon, Rowland, Thompson & Walker (7). Their values for the colostrum at parturition are in good agreement with those obtained in the present study. Direct comparison with their results for subsequent samples is difficult because Hughes & Hart give only average results for samples taken at various times between 10 and 40 hr. after parturition, while Braude *et al.* do not report samples before the tenth day of lactation.

For mineral constituents of the milk Braude *et al.* give the range of values for calcium and phosphorus for 5-day periods at three stages during the lactation. As far as comparison is possible these values also agree with the results reported here. The only analysis for magnesium content of sow's milk found in the literature is the value of 0·009 % given by Abderhalden (8), but the stage of lactation is not indicated.

Comparison with other species

The main changes during the transition from colostrum to milk—the rapid decrease in total solids and protein and increase in lactose soon after parturition—are similar to those in other species. However, in ash constituents, sow's milk appears to differ from the milk of some other species. In the cow, the ash content of colostrum is high. There is more calcium and phosphorus in the colostrum than in the milk. The amounts of these constituents decreases as the secretion becomes normal, but increases again, particularly in the case of calcium, towards the end of lactation. In the sow the concentrations of these constituents increase throughout the lactation.

SUMMARY

1. Detailed analyses for the major constituents of the colostrum and milk of four sows are reported. In all cases the initial colostrum is characterized by a very high protein, and a low lactose and fat content. As with the colostrum of other species, the transition to milk occurs progressively during the course of about 4–7 days.

2. Analyses for mineral constituents, calcium, magnesium, phosphorus and chloride are reported on a range of samples covering the whole lactation of two sows. In contrast to the ash of the colostrum of most other species, the ash of the colostrum of the sow is lower than that of normal milk, and as lactation is established there is a rise in the total ash content, and in its main constituents, calcium and phosphorus.

REFERENCES

(1) PERRIN, DAWN R. (1954). *J. Dairy Res.* **21**, 55.

(2) HAWK, P. B., OSER, B. L. & SUMMERSON, W. H. (1947). *Practical Physiological Chemistry*, 12th ed., p. 589. Philadelphia: Blakiston.

(3) HAWK, P. B., OSER, B. L. & SUMMERSON, W. H. (1947). *Practical Physiological Chemistry*, 12th ed., p. 593. Philadelphia: Blakiston.

(4) HAWK, P. B., OSER, B. L. & SUMMERSON, W. H. (1947). *Practical Physiological Chemistry*, 12th ed., p. 579. Philadelphia: Blakiston.

(5) ELSDON, G. D. (1934). *Analyst*, **59**, 665.

(6) HUGHES, H. H. & HART, H. G. (1935). *J. Nutrit.* **9**, 311.

(7) BRAUDE, R., COATES, M. E., HENRY, K. M., KON, S. K., ROWLAND, S. L., THOMPSON, S. Y. & WALKER, D. M. (1947). *Brit. J. Nutrit.* **1**, 64.

(8) ABDERHALDEN, E. (1908). *Textbook of physiological chemistry in thirty lectures*, 1st ed., p. 366. Transl. by W. T. Hall and G. Defren. New York: Wiley.

(*MS. received for publication* 29 *May* 1954)

637.17:599.5

578. THE COMPOSITION OF THE MILK OF THE BLUE WHALE

By MARGARET E. GREGORY, S. K. KON, S. J. ROWLAND and S. Y. THOMPSON

National Institute for Research in Dairying, University of Reading

During the 1950 Antarctic whaling season, samples of milk from three blue whales (*Balaenoptera sibbaldi*) were obtained for us by Mr C. E. Ash of the scientific staff of the United Whalers Ltd. factory ship *Balaena*. The samples were taken into brown bottles with screw caps when the whales, after collection by the corvettes from the catchers, were drawn up on the ramp of the factory ship. The samples were placed immediately in deep-freeze storage, kept frozen during transit later from the ship to the Institute, and returned to deep freeze until analysed in the following November.

The most extensive series of data on the composition of whale's milk has been provided recently by White (1), who reported on samples from two blue and six fin whales in terms of water, fat, protein (N × 6·38), ash and lactose. Among the earlier literature giving limited data for the milk of various species of whale may be mentioned a paper by Clowes (2) on a fin and two blue whales (and quoting a few references from 1890 onwards); one by Schmidt-Nielsen & Frog (3) on a blue, two fin and two sei whales; and one by Pedersen (4) on a humpback whale. There is, to our knowledge, no information about the individual minerals, the protein distribution, or the vitamins of whale's milk, with the exception of one value for ascorbic acid (5) in the milk of a fin whale, and one for vitamin A (6)—again a fin whale.

The examination that we have made of the three samples of whale's milk has been extended to include, as already briefly reported (7), the detailed mineral, protein and vitamin aspects.

METHODS OF ANALYSIS

Portions of each well-mixed sample were analysed by the following methods: fat by the Röse-Gottlieb method; total solids by drying at 100° C., the solids-not-fat being obtained by difference; total protein (N × 6·38) and protein fractions by the Kjeldahl method, the casein, soluble proteins and non-protein nitrogenous constituents being separated by suitable adaptation of Rowland's procedure (8); lactose by a modification of the chloramine-T method of Hinton & Macara (9); chloride by the wet digestion method of Davies (10); and ash by incineration at 500° C. On a solution of the ash, calcium was determined as oxalate, magnesium as magnesium ammonium phosphate measured colorimetrically, phosphorus as ammonium phosphomolybdate, potassium as the sodium cobaltinitrite complex and sodium as triple acetate.

Vitamin A was measured on diluted whole milk as described by Mattick, Hiscox, Crossley, Lea, Findlay, Smith, Thompson & Kon (11). For the other vitamin assays, the fat content of a part of the samples was reduced by a preliminary extraction with ether, and suitable portions were then taken, the results being calculated on the whole milk. Thiamine was determined fluorimetrically, both before and after incubation with taka-

diatase, by the method of Houston, Kon & Thompson (12) and total riboflavin by the method of Bessey, Lowry & Love (13).

Extracts for the microbiological assays were prepared as follows: riboflavin and nicotinic acid by the methods recommended by the Committee of the Society of Public Analysts (14), biotin by the method of Barton-Wright (15), and vitamin B_6 by the method of Rabinowitz & Snell (16). For pantothenic acid, the extraction was made by the method of Neilands & Strong (17) and for vitamin B_{12} (cyanocobalamin) by the sodium acetate buffer-sodium cyanide technique of Gregory (18). All the extracts were filtered, extracted with ether at pH 4·6 and adjusted to the pH of the assay medium. After extraction, the riboflavin, nicotinic acid, biotin, vitamin B_6 and pantothenic acid were assayed by published procedures, or modifications thereof, as described in an earlier paper by Coates, Ford, Harrison, Kon, Shepheard & Wilby (19); vitamin B_{12} was assayed with *Lactobaccillus leichmannii* ATCC 4797, as described by Gregory (18). All assays were made in duplicate or triplicate, and satisfactory recovery tests were carried out at the same time.

RESULTS

The samples of whale's milk were of a greyish white to pale cream colour, possessed a definitely fishy odour and had a consistency, at room temperature, resembling that of very thick cream. Sample no. 3 (which the full data suggest was abnormal) contained some blood, which gave it a faint pink tinge, and was less viscous than the other samples. The analytical results are given in Tables 1 and 2.

Table 1. *Composition of whale's milk: major constituents*

	Whale no. 1	Whale no. 2	Whale no. 3
Length of whale (ft.)	88	83	83
Date caught	10. i. 50	15. i. 50	19. ii. 50
Percentage			
Total solids	63·06	51·17	21·23
Fat	49·85	34·79	3·31
Solids-not-fat	13·21	16·38	17·92
Protein (total N × 6·38)	10·76	13·56	15·29
Lactose	0·82	1·77	0·43
Ash	1·28	1·55	1·47
Calcium	0·31	0·34	0·27
Phosphorus	0·21	0·20	0·17
Sodium	0·08	0·11	0·23
Potassium	0·13	0·16	0·08
Magnesium	0·02	0·02	0·02
Chloride	0·11	0·12	0·29
Casein (N × 6·38)	7·29	7·07	7·91
Soluble proteins (N × 6·38)	2·82	4·56	6·76
Non-protein N	0·10	0·30	0·11
Distribution of N *as percentage of total* N			
Casein N	67·8	52·1	51·7
Soluble protein N	26·3	33·6	43·9
Non-protein N	5·9	14·3	4·4

DISCUSSION

Major constituents

The values for the major constituents, in Table 1, are in agreement (except for the fat content of sample no. 3) with the previous data published for various species of whale in showing that the milk has an extremely high fat content, and, as compared with the milk of the domesticated cow, goat, ewe or sow, also a high solids-not-fat content owing to

a high protein content. The lactose content of the whale's milk, on the other hand, was much lower than that of the milk of these farm animals. As White (1) has recently indicated, in these respects whale's milk is similar to the milk of the other cetaceans and the seal, and 'rich milk of this type seems to be characteristic of mammals which live in a cold environment and whose young have a rapid rate of growth'. The high mineral content of whale's milk is also suited to this rapid growth. The calcium and phosphorus contents were particularly high—higher than in the milk of the sow (20), whose young grow very quickly, but the potassium, magnesium and chloride contents were of the same order as in the milk of the cow and goat.

Table 2. *The vitamin content of whale's milk*

	Whale no. 1	Whale no. 2	Whale no. 3
Chemical assays			
Vitamin A (i.u./g. fat)	16	18	96
Thiamine, free (μg./g. milk)	0·9	1·6	1·2
Thiamine, total (μg./g. milk)	1·2	1·6	1·3
Riboflavin (μg./g. milk)	1·2	1·3	0·4
Microbiological assays			
Riboflavin (μg./g. milk)	1·4	1·6	0·5
Nicotinic acid (μg./g. milk)	14·1	26·7	6·8
Vitamin B_6 (μg./g. milk)	1·1	1·1	0·9
Pantothenic acid (μg./g. milk)	8·5	17·7	2·7
Biotin (mμg./g. milk)	34·0	65·0	12·0
Vitamin B_{12} (mμg./g. milk)	9·0	8·0	130·0

In considering the richness of whale's milk in solids-not-fat and the separate constituents thereof, their concentrations in the fat-free milk, i.e. in the aqueous phase, and not in the whole milk with its unusually high fat content, provides the better basis of comparison with the milks of other species. On the fat-free milk basis the values given for these constituents in Table 1 would be multiplied by 2·00 for sample no. 1 and 1·53 for no. 2, whereas the corresponding factor for the milk of the cow and other domestic species is 1·03–1·08 only.

The distribution of nitrogen in the whale's milks shows a lower proportion as casein, and a correspondingly higher proportion as soluble proteins, than is found in normal cow's or goat's milk. It is not, however, possible to make a strict comparison of any aspect of the detailed composition of the whale's milks with that of the milk of other animals, as information is not available on the stage of lactation of the three whales when the samples were taken, and their milk composition will no doubt, like that of other mammals, be markedly influenced by the stage of lactation. The killing of lactating whales, i.e. those accompanied by a calf, is illegal; the samples that have been examined will have been obtained from whales accidentally separated from their calves, or whales that had recently weaned their calves and were in the last stage of lactation. The composition of sample no. 3 suggests that it may well have been of the latter type.

Vitamins

Chemical assays. The vitamin A content of the fat of samples nos. 1 and 2 was lower than that of average cow's milk, which is about 25 i.u./g. fat, but higher than that of sow's milk (11 i.u./g. fat): sample no. 3 had an unusually high vitamin A content of the fat, but this milk was low in fat, and per 100 g. of milk this sample contained 306 i.u. of vitamin A as compared with 780 and 640 i.u. in samples nos. 2 and 3. Chromatographic examination of

a sample of whale milk fat showed that, as in cow's milk (21), most of the vitamin A was in the ester form.

The thiamine content of the whale's milk was four to five times that of cow's milk (12) and more than twice that of sow's milk (20). Most of the thiamine was in the free form, and in this respect the whale's milk was similar to mid- or late-lactation cow's milk and contrasted markedly with sow's (20) and goat's (12) milk, where the alkaline phosphomonoesterase titre is low. Again, however, it is difficult to interpret some of these differences in the absence of information on the stage of lactation.

Microbiological assays. Whale's milks nos. 1 and 2 had higher biotin, riboflavin, nicotinic acid and pantothenic acid contents than no. 3. The vitamin B_6 levels were similar in all three samples, but no. 3 was higher in vitamin B_{12} than the other two. The amounts of these six B vitamins in a series of cow's and sow's milks, and of some of them in human and goat's milks, have been investigated at the Institute by the same methods of assay, and the data so obtained may be compared with the results for the whale's milks. The biotin contents of the three whale's milks were of the same order as those found for cow's milk (20–60 mμg./ml.) and sow's milk (6–30 mμg./ml.) (22). The nicotinic acid content of the whale's milks was more comparable with that found in sow's (3·8–14·9 μg./ml.) than in cow's milk (0·25–1·2 μg./ml.) (22). The vitamin B_6 level, at about 1 μg./g., was similar in all three samples. This level has sometimes been found in cow's milk, which has ranged from 0·05 to 0·97 μg./ml., but the level found in sow's milk is lower (0·1–0·3 μg./ml.) (22).

The level of pantothenic acid in samples nos. 1 and 2 of whale's milk was higher than any values found at this Institute for samples of cow's milk (1·1–5·7 μg./ml.), sow's milk (1·9–7·6 μg./ml.), human milk (1·4 μg./ml.), or goat's milk (4·8 μg./ml.) (Gregory (23)); but in sample no. 3 was within the range found for cow's and sow's milks.

The riboflavine content of whale's milks nos. 1 and 2, measured both chemically and microbiologically, was similar to that found for cow's milk (0·7–2·3 μg./ml.), sow's milk (0·9–3·5 μg./ml.) and goat's milk (1·6 μg./ml.) (23). The third sample was lower in riboflavin and more closely resembled human milk (0·35 μg./ml.) (23). The level of vitamin B_{12} was higher in all three whale's milks than is usually found in cow's milk (1·3–11·5 mμg./ml.), sow's milk (0·5–7·6 mμg./ml.), human milk (0·3 mμg./ml.), or goat's milk (0·7 mμg./ml.) (23). Sample no. 3 was particularly high in vitamin B_{12}.

SUMMARY

The detailed composition of samples of milk from three blue whales (*Balaenoptera sibbaldi*) is reported in terms of total solids, fat, solids-not-fat, total protein, casein, soluble proteins, non-protein nitrogen, lactose, chloride, ash, calcium, phosphorus, sodium, potassium, magnesium, vitamin A, thiamine, biotin, riboflavin, nicotinic acid, vitamin B_6, pantothenic acid and vitamin B_{12}.

The composition of the milk of the whale is compared with that of other species.

We wish to express our thanks to Mr C. E. Ash and United Whalers Ltd. for the help which made this study possible, and to Miss P. A. Turner and Mr A. W. Wagstaff for analytical assistance.

REFERENCES

(1) White, J. C. D. (1953). *Nature, Lond.*, **171**, 612.
(2) Clowes, A. J. (1929). *Discovery Reports*, **1**, 472.
(3) Schmidt-Nielsen, S. & Frog, F. (1933). *K. norske vidensk. Selsk. Forh.* **6**, 127.
(4) Pedersen, T. (1952). *Norsk Hvalfangsttid.* **41**, 375.
(5) Begg, M. (1947). *Nature, Lond.*, **160**, 430.
(6) Barua, R. K. & Morton, R. A. (1949). *Biochem. J.* **45**, 308.
(7) National Institute for Research in Dairying (1951). *Ann. Rep.* pp. 66 and 95.
(8) Rowland, S. J. (1938). *J. Dairy Res.* **9**, 30.
(9) Hinton, C. L. & Macara, T. (1927). *Analyst*, **52**, 668.
(10) Davies, W. L. (1932). *Analyst*, **57**, 79.
(11) Mattick, A. T. R., Hiscox, E. R., Crossley, E. L., Lea, C. H., Findlay, J. D., Smith, J. A. B., Thompson, S. Y. & Kon, S. K. (1945). *J. Dairy Res.* **14**, 116.
(12) Houston, J., Kon, S. K. & Thompson, S. Y. (1940). *J. Dairy Res.* **11**, 145.
(13) Bessey, O. A., Lowry, O. H. & Love, R. H. (1949). *J. biol. Chem.* **180**, 755.
(14) Analytical Methods Committee, Society of Public Analysts (1946). *Analyst*, **71**, 379.
(15) Barton-Wright, E. C. (1952). *Microbiological assay of the vitamin B complex and amino-acids.* London: Pitman.
(16) Rabinowitz, J. C. & Snell, E. E. (1947). *Analyt. Chem.* **19**, 277.
(17) Neilands, J. B. & Strong, F. M. (1948). *Arch. Biochem.* **19**, 287.
(18) Gregory, M. E. (1954). *Brit. J. Nutr.* **8**, 340.
(19) Coates, M. E., Ford, J. E., Harrison, G. F., Kon, S. K., Shepheard, E. E. & Wilby, F. W. (1952). *Brit. J. Nutr.* **6**, 75.
(20) Braude, R., Coates, M. E., Henry, K. M., Kon, S. K., Rowland, S. J., Thompson, S. Y. & Walker, D. M. (1947). *Brit. J. Nutr.* **1**, 64.
(21) Ganguly, J., Kon, S. K. & Thompson, S. Y. (1947). *Brit. J. Nutr.* **1**, iii.
(22) Unpublished work in progress in this Institute.
(23) Gregory, M. E. (1954). Thesis, University of Reading.

(*MS. received for publication* 23 *July* 1954)

637.128:616.9

REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION F. MILK-BORNE DISEASE

GENERAL

The increasing knowledge and importance of most aspects of dairy bacteriology are manifest from a perusal of the reviews published in this *Journal*. However, the transmission of disease to man through the agency of milk and milk products appears to have become a less frequent and less serious menace, since the papers describing outbreaks are not now so numerous as they were formerly. Furthermore, this lessening of the incidence of milk-borne disease is particularly noticeable with the infections which do not occur naturally in animals whose milk is used by mankind, though some of the natural infections of dairy cows, such as tuberculosis, have also been greatly reduced. The marked improvement in dairy hygiene and dairy techniques has lessened the opportunities for man to infect dairy animals with certain organisms. Moreover, with the lessening incidence of infectious disease in man there has been a marked reduction in the numbers of certain types of human carriers. This, together with the increasing use of pasteurization, has reduced greatly the possibility of infecting milk. In certain natural infections of milk-producing animals, the part played by infected milk in transmitting disease to humans is of less importance than direct contact with infected animals and with their secretions and excretions.

During the past few years no new books dealing exclusively with milk-borne infections have been published. A volume published under the auspices of W.H.O./F.A.O. with the title *Advances in the Control of Zoonoses* (1) contains many papers by well-known authorities and these give much up-to-date information on bovine tuberculosis, brucellosis, leptospirosis, and Q fever, and the papers, when applicable, will be referred to later. Again, in the first volume of *Recent Advances in Veterinary Science* (2) much useful information has been contributed in the chapters on animal diseases and human welfare, and veterinary public health by Meyer and Steel respectively.

TUBERCULOSIS

No great advance has been made in laboratory procedures used in the isolation, identification and typing of tubercle bacilli, but certain recently published papers are well worth perusal by those interested. Jensen (3) has again briefly outlined the methods which he employs for these purposes. Stuart (4) has described the methods employed in the Ministry of Agriculture and Fisheries Veterinary Laboratory for the examination of milk samples for tubercle bacilli. He gives details of the techniques used in microscopic examinations, biological tests, cultures and histological examinations of tissues. On account of the difficulty of obtaining guinea-pigs Gray (5) has used a double-inoculation screening test for tubercle bacilli in milk. The inoculum was the centrifuged deposit from a 100 ml. sample with, in addition, a part of the gravity cream. The inocula prepared from two samples were injected into the right and left groins of a guinea-pig, over the inguinal glands, care being taken not to enter the peritoneum. The animals were killed 6 weeks after inoculation and post-mortem examinations were made. Gray believes that with carefully kept records and good animal care the double-inoculation method permits adequate sampling and provides results which rival the single method. The results are inferior to the two-animal test only in the greater proportion of failures following premature death of the animals. The findings from most completed tests are usually clear-cut, the double negative and single positive are unequivocal. On the other hand, the apparently 'double-positive' results require careful assessment, since infection, once it has reached an advanced stage, may spread to the opposite side. Over a 7-year period the total number of animals used for biological tests was 4634, and the total number dying in 21 days or less was 551. It is suggested, therefore, that probably the best compromise of all would be to apply the double-inoculation technique to pairs of animals.

Smith (6) has studied the use of guinea-pigs, Dubos liquid and solid media, and other media in the isolation of tubercle bacilli from milk samples, the majority of which were bulk samples. He found that 10% trisodium phosphate was the most satisfactory chemical for the purpose of controlling contaminants, but this agent and 4% sodium hydroxide had a significantly lethal effect on the tubercle bacilli present. The positive results obtained on 312 samples of milk were: guinea-pig inoculation 30, culture 16, direct microscopy 10. Furthermore, acid-fast organisms were found during the microscopic examination of a further twenty-six samples, but these gave negative results on guinea-pig inoculation and on culture. These findings indicate once again the superiority of guinea-pig inoculation for the demonstration of tubercle bacilli.

The resistance of tubercle bacilli to heat when infected milk is incorporated in chocolate drinks has been studied by Bundesen, Danforth, Woolley & Lehner (7). They prepared chocolate drinks containing 12% milk and 12% ice-cream mix and subjected these to heat by the H.T.S.T. pasteurization method. They state that the use of the high-temperature short-time method requires accurate time and temperature control because a slight error in either temperature or time may have a more serious effect than when the 30 min. holding process is employed. For test purposes they employed only one strain of the bovine type of bacillus, but their experiments showed that temperatures of 160–180° F. for periods of 15–30 sec. were effective in killing the tubercle bacilli in the various preparations.

The control of bovine tuberculosis in cattle with particular reference to the methods adopted by the Ministry of Agriculture and Fisheries has been discussed by Ritchie (8). He states that in 1931 it was estimated that about 40% of cows were infected and in 1945 30–35%, when the incidence for all cattle was about 20% in England, 14% in Scotland, and 7·5% in Wales. With the establishment of many tubercle-free herds the rates of infection have now been markedly reduced, and the incidence in the country as a whole in 1952 was probably between 10 and 12%. He emphasizes that the only efficient method of detecting tuberculous cattle for the control or eradication of the disease is the tuberculin test. A survey of the presence of tubercle bacilli in milk has been made by Davis (9). He has analysed the results of guinea-pig inoculation tests for the presence of *Mycobacterium tuberculosis* in ordinary raw-accredited, ordinary bulk (Tanker) and T.T. herd, T.T. bulk (Tanker) and pasteurized milks in England and Wales for the period 1940–52. These milks showed the following percentages of positive results: (1) ordinary milk 3·4, (2) ordinary Tanker 17·5, (3) accredited 3·7, (4) T.T. herd milk 0·5, (5) T.T. Tanker 9·9, (6) holder pasteurized (145–150° F. for 30 min.) 0·16, (7) H.T.S.T. (minimum of 161° F. for 15 sec.) 0·04, (8) T.T. pasteurized both methods 0, (9) milk pasteurized by unknown methods 0·08. The author concludes that in practice T.T. pasteurized milk may be regarded as completely free from T.B., and that H.T.S.T. pasteurized milk is the safest of the ordinary pasteurized milk. The H.T.S.T. method of pasteurization is not regarded as more efficient than the holder method, but the H.T.S.T. plants are more modern in design and have more adequate controls. A full description of bovine tuberculosis in man and cattle in the various counties of Denmark and the frequency of bovine infections among urban and rural populations has been given by Jensen (10). The author states that the ingestion of bovine tubercle bacilli in milk often gives rise to many cases of latent symptom-free infections, and in those cases so infected a positive tuberculin reaction will be induced. In some districts of Denmark the eradication of tuberculosis in cattle was soon followed by a fall in the percentage of positive tuberculin reactions in children. This loss of mass immunity induced by infections with the bovine type had therefore to be made good by a comprehensive scheme of B.C.G. vaccination, and this has been in operation since 1938. The infection of cattle with the human type gives rise to a temporary positive tuberculin test, but only in rare instances can a lesion be demonstrated. Cows infected with the human type of organism do not transmit the disease to man. On the other hand, the infection of cows through the agency of individuals suffering from pulmonary tuberculosis due to the bovine type has frequently been noted, and there is a record of seventeen herds of cattle in which it had been proved that the infection was transmitted by cattlemen suffering from pulmonary tuberculosis of the bovine type. Wilson, Blacklock & Reilly (11) published a report on 'non-pulmonary tuberculosis of Bovine Origin in

Great Britain and Northern Ireland'. The report is divided into four main sections. The first is devoted to a general comparison of the findings for England, Wales and Scotland, the second describes non-pulmonary tuberculosis in England and Wales (1943–5), the third deals with meningeal and surgical tuberculosis in Scotland (1943–4) and the final section discusses human tuberculosis of bovine origin in Northern Ireland (1945–50). The English, Welsh and Scottish investigations were carried out during the war years, but the Irish work was done in the post-war years. The methods of investigation and the type of material examined varied somewhat in the

Table 1. *Distribution of human and bovine types according to country and site of lesion*

Country and years	Type of disease	H	B	Total	% B
England, 1943–5	Meningitis	183	71	254	28·0
	Surgical	550	190	740	25·7
Wales, 1943–5	Meningitis	62	7	69	10·1
	Surgical	31	12	43	27·9
Scotland, 1943–6	Meningitis	498	62	560	11·1
	Surgical	325	166	491	33·8
Northern Ireland, 1945–50	Meningitis	237	8	245	3·4
	Surgical	134	8	142	5·6

Table 2. *Meningeal and surgical tuberculosis—all ages*

	England	Wales	Scotland
No. of strains examined from all sources	994	112	1051
Percentage bovine	26·3	17·0	21·7
Percentage site of disease bovine:			
Meningitis	28·0	10·1	11·1
Cervical adenitis	57·5	44·4	67·5
Bone and joint	10·6	19·0	12·2
Abdominal	64·7	—	64·6
Genito-urinary	22·5	—	26·2
Other sites	11·8	—	9·3

different countries, so it was often difficult and sometimes impossible to make an accurate comparison. The extent of the investigation can be judged by the figures given in Tables 1 and 2, which shows the distribution of human and bovine types according to the site of the disease. In Wales and Scotland the proportion of infections due to the bovine type was higher in cases of surgical than of meningeal tuberculosis. In England the proportion of bovine-type infections in meningitis was almost double that in Scotland and treble that in Wales. Bovine infections were commoner in adult females than in adult males, and the highest rate of incidence occurred in the year period 5–10 years. In all three countries a greater percentage of those infected with the bovine type gave a history of drinking raw milk than did those suffering from an infection due to the human type. In Scotland, in particular, there was greater incidence of bovine infections in areas where there was little pasteurization and where there were many ungraded herds. The authors calculated that on the basis of the figures collected in 1944 there were in that year in Great Britain 550 deaths from tuberculous meningitis, and 1050 from other forms of non-pulmonary tuberculosis due to the bovine type of organism. In Northern Ireland the figures indicated a marked decline in bovine tuberculosis during the 10 years prior to the investigation, due principally, it was believed, to pasteurization. From 1060 strains isolated from pulmonary tuberculosis only one was of the bovine type, while the proportion of bovine infections in the non-respiratory group was 4·3% and in the meningitis group only 3·3%. It would seem likely that if a further investigation was made into the incidence of bovine-type infections now in Great Britain a very marked decrease would have occurred. The progress made in the eradication of tuberculosis in cattle has been very marked and, in addition, in urban areas in particular the extension of pasteurization must have been most helpful in this respect. Localized outbreaks of bovine tuberculosis in Great Britain have not been frequently recorded. Kind & Brough (12) have, however, investigated an outbreak of bovine tuberculosis in a rural area of Leicestershire.

Five children had clinical symptoms of infection and all obtained their milk from the same source. A series of tuberculin tests on children showed that those exposed to the risk of infection gave a much greater percentage of positive reactions than would occur in a control group of children of the same age.

INFECTIONS CAUSED BY ORGANISMS BELONGING TO THE FAMILY ENTEROBACTERIACEAE

A considerable number of organisms belonging to the genera of the Enterobacteriaceae are associated with milk-borne diseases. For as complete a classification as is possible at the present time one cannot do better than consult the new edition of Kauffmann's *Enterobacteriaceae* (13). The author points out that the Enterobacteriaceae are made up of a series of interrelated bacterial types which are not sharply divided into tribes and genera. The transition from genus to genus is gradual and intermediate strains are found. Nevertheless, the members of this vast family can be divided into tribes and genera related biochemically. Further, the genera and species can be classified serologically by utilizing their O, K and H antigens. Under the genus *Salmonella* Kauffmann lists no less than 306 types. The genera *Arizona*, *Escherichia*, *Klebsiella*, *Cloaca*, *Hafnia*, *Shigella*, *Proteus* and *Providencia* are all composed of many O groups which, in many instances, can be further subdivided by identification of H and K antigens. In fact, the classification has become so complex that reference laboratories are now essential. Weil & Saphra (14) have also published a book on the Salmonellae and Shigellae groups of organisms. This volume gives details of methods of isolation and identification, discusses questions of pathogenicity and epidemiology, and gives an account of the use of bacteriophages for diagnosis and therapy. This book is not nearly so comprehensive as that of Kauffmann.

SALMONELLA INFECTIONS

(*a*) *Enteric infections*

Few records of enteric infections due to milk have been published during the period under review. Dauer (15) in his report on food and water-borne disease outbreaks in the U.S.A. for 1951 records one small outbreak of typhoid fever in two members of one family who received their milk from a neighbour. The wife of the farmer was a carrier and all strains isolated from patients and carriers belonged to phage type F–I. In another outbreak ten cases of milk-borne *Salmonella paratyphi* B infection were traced to the nephew of a dairyman. About a week prior to the epidemic the boy had visited the dairy farm and had helped in the handling of the milk which was not pasteurized. In his report for 1952 Dauer (16) states that though there were eleven outbreaks of typhoid fever recorded none could be attributed to milk and no outbreaks of paratyphoid occurred either. The only other reports come from Germany. Staack (17) described an epidemic of 126 cases of paratyphoid fever due to a milk infection in a town with a population of 73,000 in Schleswig-Holstein. A worker in the dairy who helped to clean bottles was found to be a faecal excreter of *S. paratyphosus* B. The efficiency of the pasteurizing plant was in doubt, as the controls for time and temperature were unsatisfactory. Gaase (18) described an outbreak of paratyphoid fever which started in June 1949 in a town in western Germany, and within 6 months had involved over 400 individuals. The incidence of cases in the first few days of June was small but by the middle of the month the epidemic had assumed an explosive character. Amongst the cases which occurred in June there was a high preponderance of women and children, and this fact suggested that the disease was distributed through the agency of milk from a particular depot. The depot handled the milk from 2200 farms and was supposed to use H.T.S.T. pasteurization methods. One investigation of a group of twenty-two farm workers showed that no less than six were carriers.

(*b*) Salmonella *food-poisoning infections*

The paucity of outbreaks of *Salmonella* food poisoning is illustrated by the reports of Dauer (15, 16) on food and water-borne disease outbreaks in the U.S.A. for 1951 and 1952. In 1951 ice cream was the vehicle of infection in two outbreaks involving forty-two persons, and

from these outbreaks *S. typhi murium* and *S. oranienburg* were isolated. In 1953 milk products were again found to be the source of two small outbreaks, one outbreak being associated with ice cream and the other with egg-nog. Ten persons were involved in the first and seven in the second, the organism isolated being *S. montevideo*, and an unclassified *Salmonella*. In the United Kingdom the position has not been so fortunate, for several large outbreaks due to *S. dublin* and *S. typhi murium* have to be recorded.

In a discussion on the epidemiology and treatment of *Salmonella* infections in man and animals with special reference to *S. dublin*, Taylor (19) stated that *S. typhi murium* is the most common, being responsible for 75% of 5094 cases which occurred during 1949–51. The endogenous type *S. dublin* was only responsible for some 2%, but it seemed to cause a longer period of illness and a higher proportion of fatal cases than do the other types of food-poisoning organisms, and it was not unusual to recover the organism from the blood stream in the more severely ill cases. Lovell (20), a subsequent speaker in the same symposium, stated that *S. enteritidis* and *S. typhi murium* are catholic types, whereas *S. dublin* was particularly associated with disease in cattle. The high frequency of infection of cattle in Wales with *S. dublin* was not found in other parts of the United Kingdom. In south and west Wales 10% of 2552 samples of bile yielded *Salmonella*, most of them *S. dublin*. In tropical countries there was evidence to show that when calves were infected with plasmosis they were more prone to infection with *S. dublin*. *S. dublin* is a definite cause of specific dysentery in calves in India, East and South Africa, and in Europe. Many food-poisoning outbreaks have been due to the contamination of milk from infected animals. McCall (21, 22) also described his experience of this type of infection. Since 1947 in Somerset no less than fourteen outbreaks of food poisoning had been proved to be due to *S. dublin*, the largest milk-borne outbreak involving no fewer than 600 individuals in Chard in 1952. The causative organism was recovered from the patients, the milk and the faeces of a cow, and from the faeces of the calf of the infected cow. The only safeguard against such an infection would be efficient pasteurization. Murdock & Gordon (23) examined 1000 samples of faeces from apparently healthy cattle. An incidence of 8·6% of *S. dublin* carriers were found. On occasion also *S. thompson*, *S. gallinarum*, *S. enteritidis*, *S. typhi murium*, *S. kentucky*, and *S. oregon* were also isolated. It would appear that the incidence of bovine carriers of *S. dublin* is very high in Northern Ireland, yet the authors point out that the number of food-poisoning outbreaks due to *S. dublin* in that area is not unusual. In South Africa Henning (24) has examined a total of 507 *Salmonella* isolated from outbreaks of enteritis in calves; of these 491 were *S. dublin*, 11 *S. typhi murium*, 4 *S. enteritidis* and 1 was *S. bovis morbificans*. In addition, 12 strains of *S. dublin* were isolated from adult cattle. He has discussed the importance of the adult cow as a carrier reservoir of infection of *S. dublin* and the importance of this organism as a cause of outbreaks of milk-borne disease.

Norton & Armstrong (25) have described a large epidemic of food poisoning due to *S. typhi murium* affecting at least 252 inhabitants of Easington and its neighbourhood in County Durham. The cases occurred over a period of at least 12 days, and evidence was obtained that the vehicle of dissemination was infected milk from a herd of 150 tuberculin-tested cows. A sample of the herd milk gave negative results, but information was received that one animal was ill and from the milk and faeces of this animal *S. typhi murium* was obtained. No further animals in the herd were found to harbour the organism, but it was obtained from the faeces of fifteen out of twenty-three milk handlers and from nine out of sixty-five members of milk-handlers' families. The possible sources of contamination were, therefore, very numerous. The strains of *S. typhi murium* isolated from patients, contacts, and cow all belonged to phage type 1*a*. As soon as the milk was pasteurized the number of notifications fell rapidly, but the restraint order on the sale of raw milk was maintained for 5 weeks, by which time the milk handlers and the cow were free from infection. Similarly, Riddell (26) described a large outbreak of food poisoning which occurred in the county of West Midlothian in Scotland and in which the causative agent was *S. typhi murium*. It was estimated that there were 211 cases of which 123 occurred in school and pre-school children. The cases were distributed over a period of 8 days with the maximum incidence on the 3rd, 4th and 5th when 51, 52 and 44 individuals developed the disease. The outbreak

had been under way for 3 days before being brought to the notice of the Medical Officer of Health. The distribution of the disease suggested that it was milk-borne, although no absolute proof was obtained. The original source of the infection was either direct contamination of a T.T. milk supply by infected cattle or through the agency of an infected dairy worker. At the time of the occurrence the plant at the creamery which supplied the milk was undergoing rearrangement and renewal, and the evidence indicated that the T.T. milk supply was first infected and that part of the pasteurized supply was contaminated in a bottling machine which had been used for bottling the T.T. milk. Within 3 days of the first contamination the T.T. milk supply was discontinued and milk from another source was substituted. This had been arranged for by the creamery in the course of business and was not due to knowledge of the outbreak, and consequently this probably lessened the spread of the infection. After the outbreak had ceased reports were received of the occurrence of *S. typhi murium* infections in cows and calves on several farms in the district and also of infections in rats caught on the farms.

SHIGELLA INFECTIONS

Only one brief account of milk-borne dysentery can be traced in recent literature. Dauer (16), the Chief Medical Adviser to the Department of Statistics of the U.S. Public Health Service in his annual report for the year 1952, described a large outbreak of milk-borne Sonne dysentery. In one particular school 639 children were affected. Epidemiological investigations revealed that pasteurization had not been carried out properly. The source from which the milk was infected was not traced.

ESCHERICHIA COLI INFECTIONS

The serological classification of the *Escherichia coli* group of the Enterobacteriaceae has been placed on a firm basis by Kauffmann (27), and now there are 128 'O' groups with further subtypes associated with their H and K antigens. In recent years also certain specific types of *E. coli*, O 25, O 26, O 55, O 86, O 111, O 114 and O 119, have been found to be particularly associated with infantile diarrhoea and vomiting in various parts of the world (Smith (28)). In fact, the epidemiological evidence, and the correlation between types and outbreaks, leaves no doubt that certain forms of infantile gastro-enteritis must be due to these specific types.

An interesting development has been the serological classification of strains of *E. coli* obtained from calves by Wramby (29) and the correlation of animal types with human types by Bokhari & Ørskov (30). Furthermore, the problem of 'white scours' in calves has been reinvestigated by workers in the Department of Pathology of the Royal Veterinary College and in the National Institute for Research in Dairying (31, 32). They have shown that the disease is due to an infection with certain types of *E. coli*, and that it occurs, to begin with, in calves from whom the protective colostrum has been withheld, but in epidemic condition it may be transmitted to calves which have had colostrum. These workers have also shown that it is essential for the colostrum to contain specific antibodies in order to give protection. Strains O 26, O 86 and O 119, which have been isolated from cases of infantile gastro-enteritis, are also associated with cases of 'white scours'. No evidence has been offered, so far, that the disease of calves can be transmitted to bottle-fed infants through the agency of infected milk, though this seems quite a possibility. Another feature of the epidemiology of the disease in infants is its association with bottle rather than breast-fed infants. Immunity in the human infant is transmitted from the mother through the placental circulation, and the difference in susceptibility between the breast and bottle-fed baby may be that the latter is exposed to a greater risk through infected milk. In connexion with the possible contamination of milk with these organisms Fey (33) examined by serological methods 113 strains of *E. coli* obtained from mastitis in cows. Three-quarters of these belonged to 'O' groups 2, 68, 9, 21, 81 and 86. The O 86 type has been found by Taylor & Charter (34) to be associated with infantile gastro-enteritis, and, furthermore, Fey has also recovered one strain of O 55 and one of O 26 from cases of mastitis, while he was able to isolate one strain of O 111 from

the prepuce of a bull. At the farm on which the cow with the O 26 mastitis was found specimens of faeces were obtained from the farmer's family, and those from the wife and three children contained *E. coli* O 26, though none gave a history of any intestinal upset.

DIPHTHERIA, STREPTOCOCCAL AND STAPHYLOCOCCAL INFECTIONS

During the past two years there does not seem to have been any recorded outbreak of milk-borne diphtheria or streptococcal infection. This must be an unusual occurrence and must indicate that *Corynebacterium diphtheriae* and group A haemolytic streptococci are less frequently implanted by man in the cow's udder, or if contamination does occur then pasteurization must be an effective safeguard.

There have also been few staphylococcal milk-borne outbreaks of any importance. The epidemiological investigation of such outbreaks is still dependent on phage typing. Allison (35) pointed out that phage type 6/47 (group III) was particularly associated with food poisoning. This work has been confirmed and extended by Williams and his colleagues (36) in England, who found that in outbreaks of food poisoning associated with various foodstuffs staphylococci belonging to group III phage types were frequently present, whereas staphylococci belonging to phage groups I and II were rarely found. In thirty-five out of forty outbreaks in which the data were complete the organisms fell into group III, while in forty-one outbreaks where the evidence of peccancy was not so complete 78% were found to be associated with organisms of the same phage group. The authors note that there is, as yet, no estimate of the proportion of strains of group III that produce enterotoxin and that a strain cannot be classified as a food-poisoning type simply because it belongs to phage group III.

Dauer (15, 16) reported that in the U.S.A. in 1951 and 1952, there were sixty-three and seventy-seven outbreaks of staphylococcal food poisoning notified, and of these eighteen and fifteen respectively were associated with cream-filled pastries. In only three instances in 1951 and in no instance in 1952 was milk incriminated as the vehicle of infection. Most of the outbreaks occurred in residential schools, eating places, and after banquets. Bravo (37) described an outbreak of staphylococcal food poisoning due to infected ice cream which affected eighty-six individuals out of a total of 148 in four family reunions. Within 2–4 hr. of eating the peccant material most individuals affected complained of epigastric pain and repeated vomiting, followed later by some diarrhoea. Ice cream was the only common food used by all four families, and *Staphylococcus aureus* was cultured from a sample obtained in one household. Two of the workers at the ice-cream factory showed *Staph. aureus* in their pharyngeal secretion and a similar *Staphylococcus* was also cultured from four out of the many containers of ice cream at the factory.

BRUCELLOSIS

The laboratory methods used in the detection of brucellosis by the Veterinary Laboratory of the Ministry of Agriculture and Fisheries at Weybridge have been fully described (38). These include the technique for the isolation of the organism from infected material, differential methods of typing including the preparation of *Brucella abortus* and *Br. melitensis* monospecific antisera, methods of carrying out agglutination tests including ring tests, and the preparation of vaccines with strain 19. Castaneda (39) has also reviewed the various tests used for the diagnosis of human brucellosis, and suggests that where it is not possible to carry out repeated blood cultures and agglutination tests, the spot test, the surface-fixation test, and the skin test should be applied. The primary object of the spot test is to obtain a positive-slide agglutination test when antibody is present in the serum in a titre of 1 to 100 or more. A suspension of *Br. melitensis* stained with methylene blue is used as the antigen, and the test may be performed with whole or citrated blood. The surface-fixation test is performed in a manner similar to the technique used in paper chromatography: the haematoxylin-stained antigen is placed in drops on the filter-paper, the serum under test along with positive and negative control samples are each dropped on the dots of stained antigen, and then a current of saline is allowed to cover the paper by capillary attraction.

In positive fixation tests the stained spot remains fixed but in negative tests the spots will be washed out. For the skin test a hapten-like soluble extract of brucellae has been prepared which, properly standardized, has been found to be quite satisfactory for the detection of skin sensitivity in human brucellosis. Cruickshank(40), as Director of the Brucella Reference Laboratory, has reported on the examination of 800 strains of *Brucella* species. Most of the strains were submitted from the laboratories of the Public Health Laboratory Service in England and most were isolated from milk, but some were from cases of undulant fever. The chief tests applied were CO_2 requirements, H_2S production, growth in the presence of four dyes, and agglutination by monospecific antisera. Of 738 strains from milk in Britain 680 were *Br. abortus*, and of these sixty-two were of a type inhibited by all the usual test dyes in their customary concentrations; thirty strains of *Br. melitensis* were obtained from twenty-four separate farms and one tuberculin-tested bulk supply; eighteen strains behaved like *Br. abortus* in biochemical tests, but like *Br. melitensis* in their serological reactions. Of the strains isolated from human infections in this country all, with two exceptions, were *Br. abortus*. The general conclusion was reached that the tests give consistent results and enable strains to be classified satisfactorily for epidemiological purposes.

Stableforth(41) has described in detail the prevalence of animal brucellosis, the species of *Brucella* with their differentiation and distribution, methods of diagnosis, and the control of the disease by eradication and by vaccination. The prevalence of infection in animals in European countries varies from 10 to 30%, while in the U.S.A. 4% of cows and 16% of herds are infected. In Great Britain it is more difficult to give an accurate estimate, as the position has been complicated by vaccination with strain 19. In Great Britain also the policy of vaccination with strain 19 was adopted at the beginning of the Second World War, and as a result, though the percentage of infected herds is not known, brucellosis is not now regarded as such a serious economic problem. Ferguson & Robertson(42) have made an evaluation of the milk-ring test in a survey of the incidence of bovine brucellosis in southern Scotland. A total of 438 simultaneous blood-serum and milk-ring tests were made, and they found that the ring test was correct in some 93% of cases in detecting cows whose blood-serum tests were also positive. The ring test could, therefore, be used as a simple time-saving and accurate screening test for detecting herds possibly infected with *Brucella*. At least 10·5% of herd raw-milk samples in Edinburgh were shown to contain *Br. abortus* when tested by guinea-pig inoculation. Antigens stained by tetrazolium or by haematoxylin were equally effective when employed in the test.

Feig(43) has given a detailed account of brucellosis in Iowa, Minnesota and Wisconsin. The reported incidence in the U.S.A. reached a peak in 1947 with 6321 cases and declined to 2196 cases in 1951, giving morbidity rates of 4·9 and 1·9 respectively per 100,000 of the population. The three Mid-West States contributed 25% of all cases during the 10-year period 1942–51 and 50% of all cases reported in 1951. Except among meat handlers the method of transmission was not easy to determine. 90% of Wisconsin's male farm cases consumed their own unpasteurized milk, but 40% of urban cases claimed to have consumed nothing but pasteurized milk. In general four times as many males as females became ill, while the age distribution of 806 cases showed 4·7% under 10 years, and 70% between 20 and 49 years. The case fatality rate was low, 1·5% nationally but only 0·2% in Minnesota, 0·3% in Iowa, and 0·7% in Wisconsin. Renoux(44) has reviewed the various aspects of human brucellosis, such as the route of transmission from animal to man, the course of the disease in man, therapy, economic importance, and the control of human infections. The author remarks that the ingestion of milk or milk products is often given as the principal mode of transmission, but this route is neither the only one, nor even the principal one. In France it has been shown that infection with *Br. melitensis* is a disease for the most part of people living in villages and the country, and the victims are usually those who handle infected manure and have direct contact with infected animals. It is mostly in towns that infection by ingestion is more evident. Direct contact infection as well as ingestion is also important in the transmission of disease due to *Br. abortus*. In countries where small ruminants are predominantly used for producing milk then the infection is transmitted through ingestion of

raw milk and milk products. *Br. suis* infections, which produce a serious illness in man, can also be transmitted by contact and by ingestion. The author estimates that the economic loss to farmers and ill-health among the population costs France 37,000,000,000 francs per annum. Various regulations are suggested for the control of the disease, and much emphasis is placed on an extensive educational programme. A special study was made by Spink(45) of the problem of brucellosis, due to *Br. melitensis*, in visits to Mexico City, Montpellier, Florence, Rijeka in Yugoslavia, and to Spain. In France and Italy, as elsewhere, the disease develops in rural communities living in close contact with goats and sheep, though cattle and swine may also harbour the infection. It reflects a high degree of contact of human beings with infected animals and their environment. Even in the Infectious Diseases Hospital in Madrid in October 1952, there were no less than thirty cases of brucellosis, and those affected accounted for 10% of the hospital population. In Yugoslavia in 1947 it was recognized that an epidemic of brucellosis was occurring amongst the rural population in the district of Rijeka, since in that year 122 patients were treated for this disease in the fever hospital. In the succeeding years 1948, 1949 and 1950, there were 176, 110 and 24 cases. The source of infection was traced to sheep, and the occurrence of the disease in sheep was attributed to the mingling of native animals with infected Italian flocks which had been driven into Yugoslavia during the Second World War in order to prevent their confiscation by the retreating German army. Tests showed 20% of the sheep to be infected and no less than 10,000 were slaughtered. This drastic procedure was believed to have accounted for the reduction of the incidence in human beings. The same author(46) has reported on 204 cases of brucellosis which he had seen in the clinics of the University of Minnesota between the years 1937 and 1953. Of these 204 patients, 119 had positive blood cultures and the types of organisms isolated were *Br. abortus* 102, *Br. suis* 12 and *Br. melitensis* 5. While *Br. abortus* is the least invasive it causes approximately three-fourths of the cases in human beings in the United States. Of the 204 patients, 153 were men, mostly between the ages of 20 and 40 years. Information on the mode of infection suggested that contact with infected animals and their contaminated environment may have been just as important as the ingestion of raw milk. In only one family was there unqualified evidence that the ingestion of raw milk was the sole source of the disease. This family study emphasized a common feature of communicable disease in that a considerable number may be exposed to infection, the tissue of many will have been invaded by micro-organisms, but relatively few will manifest evidence of illness. Barrett & Rickards(47, 48) have discussed the clinical syndrome of chronic brucellosis in a series of twenty-five individuals. They believe that apart from the isolation of the infecting organism there is no single criterion upon which the diagnosis can be established. Cases often give negative serum-agglutination tests, and in some of these agglutinins will appear following upon an intradermal test with brucellin. Fourteen out of the twenty-five individuals who were under observation had been directly connected with farms or with the marketing of dairy products. Direct contact with infected animals or infected premises seemed to be more important than the drinking of raw milk.

Gillmann(49) described a case of undulant fever in a veterinary worker due to *Br. abortus* strain 19, following upon accidental inoculation. More recently, Spink(50) reported two cases of undulant fever due to strain 19 which has now been successfully used for immunization of cattle. Again both were in veterinary workers, and neither was contracted through the agency of infected milk but both were due to mishaps with syringes containing living suspensions of the organisms. The author of this review has had a similar experience in which a veterinary officer accidentally pricked his finger with the needle of a syringe containing the living vaccine prepared from strain 19. It would appear, therefore, that this strain, though apparently suitable for immunizing cattle, is not innocuous for human beings. So far as is known no human infections have been contracted through the agency of milk infected with this organism, and illness has occurred only when accidental inoculation has taken place.

LEPTOSPIROSIS

Leptospirosis, in its different forms, is now recognized as a disease with a world-wide distribution. The causative organism can be harboured by many different animals, and man contracts infection at times directly from animal sources, and at other times indirectly by contact with most environmental conditions in which pathogenic leptospira have survived after having been deposited there by infected animals.

The laboratory diagnosis of leptospirosis has been described in detail by Wolff(51). He has given a full description of microscopic techniques, cultural procedures and animal and serological tests. In another paper Wolff(52) has also reviewed the serological classification of type strains. This classification has been further amplified by Wolff & Broom(53). In the introduction to this valuable paper the authors have reviewed the history of the isolation of *Leptospira icterohaemorrhagiae*, *L. hebdomides*, *L. autumnalis* and *L. biflexa*, and have described the various tests used in identifying these organisms. They then discuss the value of the methods of differentiation as applied to the genus as a whole and express the opinion that pathogenicity, protection, and Pfeiffer's tests which are carried out *in vivo* are subject to considerable variation due to changes in the virulence of the organism and in the resistance of the host. The *in vitro* tests, such as agglutination-lysis, agglutinin-absorption and complement-fixation, are more suitable since they are subject to less change. Stability of the antigenic structure is essential if serological tests are to be applied, and this they believe to be inherent in all strains of leptospires. A review of the history of abnormal serological findings associated with so-called strains of *L. icterohaemorrhagiae* in particular suggests that the variations in antigenic structure described by various workers were due in some cases to mistakes in labelling and in other instances to the possibility of cultures being contaminated with water *Leptospira* which overgrew and finally destroyed the original strain. Methods for avoiding mistakes in subculturing are given in detail. The authors then propose that the members of the group should be divided into sero-groups and sero-types, then when two or more sero-types show marked similarity in their serological relationship they should be assembled under one sero-group. Two strains are considered to belong to different sero-types if after cross-absorption with adequate amounts of heterologous antigen 10% or more of the homologous titre regularly remains in each antiserum. In order to avoid difficulties in classification full details are given of the application of agglutination-lysis tests, agglutinin-absorption tests, notation of results, agglutination diagrams, and designation of antigenic factors. A list is given of thirty-two sero-types which have been established so far, and these authors suggest that further sero-types should not be added until full comparison has been made with the strains available in the collection at Amsterdam or in collections in other centres as they are established.

Many papers have been published describing human cases of infection with *L. pomona*, *L. grippo-typhosa* and *L. mitis*. In all these the source of infection was attributed to direct contact with infected animals such as cattle, sheep, pigs and goats, and not to the ingestion of infected milk.

Q FEVER

Q fever has now been recognized and has been reported as occurring in Europe, Africa, Asia, Australasia, Central and North America. Kaplan & Hulse(54) have described the incidence, so far as it has been investigated, of Q fever in the countries of Europe and the survey methods employed in its detection. This record includes what is known about the occurrence of actual cases, the incidence of positive sero-reactions in groups of human individuals, and the distribution of the disease in various animals. The countries which appear to be the most affected are Italy, Switzerland and Greece, while no reports have been obtained of positive results in Denmark, Sweden and Iceland. A series of papers have been published on infection of animals and man in Great Britain, particularly in England. Slavin(55) examined by the complement-fixation test blood samples from guinea-pigs inoculated with 2581 milk samples from England, 553 from

Wales and 240 from Scotland. These results showed 6·9% of infected farms in England, 2% in Wales and 0·8% in Scotland. Samples of blood from 3767 cattle showed a significant antibody titre in 2%. Samples from one large dairy plant demonstrated clearly the efficiency of pasteurization, when tests showed that *Rickettsia burnetti* could be quite easily recovered from 3000-gallon tankers and that pasteurization completely eliminated tubercle bacilli and *R. burnetti*. Stoker & Thompson (56) described a third outbreak of Q fever, distinguished by a high attack rate, which took place in September 1951, amongst residents and visitors at a farm in south Kent. Thirteen out of fifteen people on the farm and three out of nine visitors contracted the disease, and in addition one child had a subclinical infection. No laboratory evidence could be obtained, however, of *Coxiella burnetti* infection in the cows, and although there was some evidence that pigs might be the source it was not possible to confirm this. Stoker (57) has reviewed the literature on the prevalence of Q fever in Great Britain. He states that case-to-case infection amongst human is extremely rare, and that the bulk of human infections are derived from infected domestic animals which remain well but which, at parturition, excrete large numbers of organisms in the placenta and faeces and thereafter in the milk. With more knowledge about the relative roles of milk and air-borne infection, it may be possible to cut down the opportunities for transmission of this infection to man. Marmion *et al.* (58) gave an analysis of sixty-nine sporadic cases of Q fever in Great Britain with a study of the prevalence of infection of human beings and cows. They have summarized the information about those outbreaks previously described by Harman (59), Harvey, Forbes & Marmion (60) and by Stoker & Thompson (56). Excluding these outbreaks sixty-nine sporadic cases have been identified since 1948; fifty-eight of these had a clinical syndrome resembling atypical pneumonia and four died. Thirty-two were infected in Kent, and the remaining cases were scattered over England and Wales. Two-thirds of the patients gave a history of exposure to known sources of Q fever. Of 4407 normal blood specimens examined 2·13% gave evidence of previous infection. Examination of the milk from 1031 herds of cattle showed 8·2% of the samples from herds in Kent contained *R. burnetti* compared with 2·7% in Devon and 0·85% in East Anglia. In a further paper Marmion, Stewart, Richmond, Barber & Stoker (61) have described the part played by sheep in the transmission of the disease to man, with special reference to the Romney Marsh area of Kent. In this area specimens of blood were obtained from five flocks of sheep on farms where human cases had occurred. The sera from these animals gave positive complement-fixation tests at 1/10 or more in 4·3, 2·3, 33·7, 1·6 and 7·7% of specimens from each farm. The authors point out that the seasonal incidence of human cases is greatest during the months of February to May at the time of lambing and when sheep are being castrated, 'ducked', shorn and generally much handled. They found no evidence that cattle were commonly infected, and, furthermore, the consumption of raw milk could only have accounted for three out of ten sporadic human cases.

Babudieri (62) has discussed the epidemiology, diagnosis and prophylaxis of Q fever. The distribution of the disease is noted and the author states that in 1950–1, in Italy alone, it was estimated that there were 20,000 human infections in a total population of 47 millions. The disease, apparently, produces little or nothing in the way of illness in animal vectors and in consequence is of little interest to veterinarians. The organismal cause, *Coxiella burnetti*, has a remarkable resistance to physical and chemical bactericidal agents. It can withstand a temperature of 50° C. for at least 30 min. and temperatures of 60 and 70° C. for shorter periods. The disease is transmitted to man by direct contact with infected secretions and excretions, by infected airborne droplets and dust, and by the ingestion of infected milk and milk products. Infection has been found to be extensive in cattle, sheep and goats and even occurs in horses, pigs and dogs. Certain animals may become infected before lactation starts and may begin to eliminate the coxiellae when giving their first milk. Even infected women if lactating can also excrete the organism in their milk.

So far as infections due to the ingestion of raw milk and milk products are concerned the only suitable preventative measure is effective pasteurization at temperatures higher than is normally employed at present. The author states that various Italian workers have shown that

low-temperature pasteurization is not enough and that the milk should be heated to 80° C. for 1 min. Everything that has been said about milk applies to unheated milk products except cheese. In this foodstuff the coxiellae usually die out within 30–40 days of its preparation.

VIRUS DISEASES

In view of the suggestion that certain epidemics of poliomyelitis may have been caused by infected milk, Kaplan & Melnik (63) thought it important to find out whether the American official minimum temperatures required for the pasteurization of milk (61·7° C. or 143° F. for 30 min. or 71·1° C. or 160° F. for 15 sec.) were adequate for the destruction of various strains of the virus in milk. The viruses subjected to test were obtained from human faeces and an infected spinal cord, together with a rodent strain (Y–SK) and the Lansing strain. The techniques of the preparation and heating of specimens are given in detail. Under these experimental conditions the virus as obtained from human faecal material was inactivated by the holding and high-temperature short-time method of pasteurization of milk, cream and ice cream, even although the materials were contaminated with quantities of virus which were much in excess of what would occur naturally. On the other hand, the virus which had been adopted to cotton rats was inactivated by the holding method of pasteurization but not by the high-temperature short-time method when suspended in cream. In cream the Lansing strain required to be heated for 45 sec. and the Y–SK strain 180 sec. before inactivation was complete. The fatty material delayed the penetration of sufficient heat to destroy the virus.

J. SMITH

REGIONAL LABORATORY
CITY HOSPITAL
ABERDEEN

REFERENCES

(1) *Advances in the control of zoonoses* (1953). Geneva: W.H.O.
(2) *Recent advances in veterinary science* (1953). Vol. **1**. New York: Academic Press Inc.
(3) JENSEN (1953). *Advances in the control of zoonoses*, p. 35. Geneva: W.H.O.
(4) STUART (1953). *Advances in the control of zoonoses*, p. 39. Geneva: W.H.O.
(5) GRAY, EVANS & PEARCE (1951). *Mon. Bull. Minist. Hlth Lab. Serv.* **10**, 284.
(6) SMITH (1953). *J. Path Bact.* **65**, 67.
(7) BUNDESEN, DANFORTH, WOOLLEY & LEHNER (1953). *Amer. J. publ. Hlth*, **43**, 185.
(8) RITCHIE (1953). *Advances in the control of zoonoses*, p. 25. Geneva: W.H.O.
(9) DAVIS (1953). *Riassunti delle Communicazioni, VIth Congresso Internationale di Microbiologia, Roma*, **3**, 211.
(10) JENSEN (1953). *Advances in the control of zoonoses*, p. 11. Geneva: W.H.O.
(11) WILSON, BLACKLOCK & REILLY (1952). *Non-pulmonary tuberculosis of bovine origin*, p. 5. London: N.A.P.T.
(12) KIND & BROUGH (1954). *Brit. med. J.* **1**, 372.
(13) KAUFFMANN (1954). *Enterobacteriaceae*, p. 7. Copenhagen: Munksgaard.
(14) WEIL & SAPHRA (1954). *Salmonellae and Shigellae*. Illinois, U.S.A.: Thomas.
(15) DAUER (1951). *Publ. Hlth Rep.* **67**, 1089.
(16) DAUER (1952). *Publ. Hlth Rep.* **68**, 696.
(17) STAACK (1953). *Arch. Hyg., Berl.* **137**, 355.
(18) GAASE (1953). *Z. Hyg. Infekt.* **137**, 177.
(19) TAYLOR (1953). *Proc. Roy. Soc. Med.* **46**, 445.
(20) LOVELL (1953). *Proc. Roy. Soc. Med.* **46**, 445.
(21) MCCALL (1953). *Proc. Roy. Soc. Med.* **46**, 447.
(22) MCCALL (1953). *Lancet*, **1**, 1302.
(23) MURDOCK & GORDON (1953). *Mon. Bull. Minist. Hlth Lab. Serv.* **12**, 72.
(24) HENNING (1953). *Onderstepoort J. vet. Rec.* **26**, 3.
(25) NORTON & ARMSTRONG (1954). *Mon. Bull. Minist. Hlth Lab. Serv.* **13**, 90.

(26) Riddell (1954). *Hlth Bull.* **12**, 34.
(27) Kauffmann (1947). *J. Immunol.* **57**, 71.
(28) Smith (1953). *J. Path. Bact.* **64**, 503.
(29) Wramby (1948). *Acta path. microbiol. scand.* Suppl. p. 76.
(30) Bokhari & Ørskov (1952). *Acta path. microbiol. scand.* **30**, 87.
(31) Briggs, Lovell, Aschaffenburg, Bartlett, Kon, Roy, Thompson & Walker (1951). *Brit. J. Nutr.* **5**, 356.
(32) Ingram, Lovell, Wood, Aschaffenburg, Bartlett, Kon, Roy & Sears (1952). *Proc. XIII Int. Dairy Congr. The Hague*, **3**, 1365.
(33) Fey (1953). *Riassunti delle Communicazioni, VIth Congresso Internazionale di Microbiologia, Roma*, **2**, 308.
(34) Taylor & Charter (1952). *J. Path. Bact.* **66**, 715.
(35) Allison (1949). *Proc. Roy. Soc. Med.* **42**, 214.
(36) Williams, Rippon & Dowsett (1953). *Lancet*, **1**, 510.
(37) Bravo (1953). *Riv. Ecuatoriana de Hig. y Med. Trop.* **10**, 13.
(38) Staff Minist. Agric. and Fish. Veterinary Lab. (1953). *Advances in the control of zoonoses*, p. 89. Geneva: W.H.O.
(39) Castenada (1953). *Bull. W.H.O.* **9**, 399.
(40) Cruickshank (1954). *J. Hyg., Camb.*, **52**, 105.
(41) Stableforth (1953). *Advances in the control of zoonoses*, p. 71. Geneva: W.H.O.
(42) Ferguson & Robertson (1954). *J. Hyg., Camb.*, **52**, 24.
(43) Feig (1952). *Amer. J. Publ. Hlth*, **42**, 1253.
(44) Renoux (1953). *Advances in the control of zoonoses*, p. 61. Geneva: W.H.O.
(45) Spink (1953). *Bull. W.H.O.* **9**, 385.
(46) Spink (1954). *Amer. J. Med. Sci.* **227**, 128.
(47) Barrett & Rickards (1953). *Quart. J. Med.* **22**, 23.
(48) Barrett & Rickards (1954). *Lancet*, **1**, 294.
(49) Gillman (1944). *Cornell Vet.* **34**, 193.
(50) Spink & Thompson (1953). *J. Amer. med. Ass.* **153**, 1162.
(51) Wolff (1953). *Advances in the control of zoonoses*, p. 127. Geneva: W.H.O.
(52) Wolff (1953). *Advances in the control of zoonoses*, p. 137. Geneva: W.H.O.
(53) Wolff & Broom (1954). *Documenta de Medecina Geographica et Tropica*, **6**, 77.
(54) Kaplan & Hulse (1953). *Advances in the control of zoonoses*, p. 175. Geneva: W.H.O.
(55) Slavin (1952). *Vet. Rec.* **64**, 743.
(56) Stoker & Thompson (1953). *Lancet*, **1**, 137.
(57) Stoker (1953). *Brit. med. Bull.* **9**, 231.
(58) Marmion (1953). *Lancet*, **2**, 616.
(59) Harman (1949). *Lancet*, **2**, 1028.
(60) Harvey, Forbes & Marmion (1951). *Lancet*, **2**, 1152.
(61) Marmion, Stewart, Richmond, Barber & Stoker (1954). *Lancet*, **1**, 1288.
(62) Babudieri. *Advances in the control of zoonoses*, p. 157. Geneva: W.H.O.
(63) Kaplan & Melnick (1952). *Amer. J. Publ. Hlth*, **42**, 525.

(*MS. received for publication* 3 *September* 1954)